Cognitive and Brain Structural Effects of Long-Term High-Effort Endurance Exercise in Older Adults: Are There Measurable Benefits?

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Statement

I hereby declare that this thesis has not been previously submitted, in whole or in part, to this or any other University for a degree.

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Summary

Age-related decline in cognitive performance and brain structure can be offset by increased exercise. Little is known, however, about the cognitive and brain structural consequences of long-term high-effort endurance exercise. In a cross-sectional design, we recruited older adults who had been engaging in high-effort endurance exercise over at least twenty years, and compared their cognitive performance and brain structure with a non-sedentary control group similar in age, sex, education, IQ, depression levels, and other lifestyle factors. We hypothesized that long-term high-effort endurance exercise would protect against the age-related decline in memory, attention, and brain structure. Our findings, in contrast to previous studies, indicated that those participating in long-term high-effort endurance exercise, when compared without confounds to non-sedentary control volunteers, showed no differences on measures of speed of processing, executive function, incidental memory, episodic memory, working memory, or visual search. On measures of prospective memory, long-term exercisers performance suggested a self-imposed increase in effort, which did not impact on ability to complete the PM task. In complex attention tasks, they displayed a differential strategy to controls. Structurally, long-term exercisers only displayed higher diffuse axial diffusivity, an index of axonal integrity, than controls, but this did not correlate with any cognitive differences.

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A. Introduction and Overview

A.1 Theoretical Considerations

A.1.1 Aging and Cognitive Function

As we age, our cognitive abilities decline: older adults have more difficulty than younger adults with tasks that are more complex, effortful, and strategic (for reviews see Luo & Craik, 2008; Salthouse, 2010). There are many explanations for why these cognitive changes happen, and they can be summarized as: a slowing of processing speed (Salthouse, 1996, 2000); reduced attentional processing resources (Craik, 2006; McAvinue et al., 2012); loss of inhibitory functions (Darowski, Helder, Zacks, Hasher, & Hambrick, 2008; Peltsch, Hemraj, Garcia, & Munoz, 2011; Zanto, Rubens, Thangavel, & Gazzaley, 2011); and decline in controlled processing (Coubard et al., 2011).

The theory of slowing processing speed comes from path analysis, where Salthouse (1996) observed that after controlling for processing speed, the contribution of age to many cognitive functions was weak. Luo & Craik (2008) however point out that allowing unlimited processing time does not fully compensate for age-related decline in the performance of older adults, but instead younger adults show more improvement (Bryan & Luszcz, 1996; Craik & Rabinowitz, 1985; Rabinowitz, 1989). Since older adults do not seem to be achieving comparable levels of performance despite using additional time, there must be more factors than slowing that cause age-related deficits.

Then in addition to requiring more processing time, it is likely that older adults may have reduced attentional processing resources and this contributes to their inability to use this extra time effectively. To this end, Craik & Byrd (1982) were able to mimic the effects of age with younger adults by having them do a parallel secondary task, thereby reducing the younger adults' attentional processing resources. In older adults less attentional resources can also lead to information being encoded less distinctively leading to worse recall performance than

younger adults, as demonstrated for example in a face-name divided attention paradigm (Naveh-Benjamin, Guez, Kilb, & Reedy, 2004). Also the fewer attentional resources in older adults has been observed to lead to deficits in integrating multiple pieces of information (Salthouse & Mitchell, 1989).

Related to age-related change in capacity of attentional resources, older adults may also demonstrate less efficient inhibitory control (Hasher & Zacks, 1988). Inhibitory control is also termed interference control and distraction control. Evidence for this comes from Darowski et al. (2008) using path analysis finding that interference control, as measured by high-distraction reading time (Connelly, Hasher, & Zacks, 1991), partially mediated the relationship between age and higher-order cognition, measured by working memory and matrix reasoning (Raven, 1965) tasks. More recently, using a saccade and anti-saccade paradigm, Peltsch et al. (2011) observed that the ability to inhibit automatic responses, in their case an automatic saccade and instead making an anti-saccade, indeed decreases with age. Functionally, using fMRI and an alternating target letter task, Nielson, Langenecker, & Garavan (2002) observed that older adults had poorer inhibitory control and greater prefrontal activation than younger adults and activated additional areas including prefrontal areas to compensate. Using a Stroop task (Stroop, 1935) and fMRI, Langenecker, Nielson, & Rao (2004) observed greater activation in older adults than younger adults in prefrontal areas. Similarly, Mathis, Schunck, & Erb (2009) also observed greater activation in parietal and prefrontal areas as well as recruitment of additional brain areas in older adults. Nielson et al. (2002) speculated that the increased activation and recruitment of prefrontal areas is due to diffuse prefrontal losses in older adults. And indeed using DTI measures with the Stroop task, Wolf et al. (2013) recently observed that better performance (i.e. less Stroop interference) was associated with better white matter integrity of frontal pathways and they suggested that this may mediate the relationship between age and inhibitory control.

The theory of age-related decline in controlled processing combines the previous two models and defines age-related change in terms of changes in automatic and controlled processing. Controlled processing has also been termed intentional processing and effortful processing. Jacoby (1991) differentiated familiarity (automatic processing) from recollection (controlled processing) with his processing dissociation procedure. Using this paradigm with younger and older adults, Jennings & Jacoby (1993) demonstrated that in older adults automatic processing, as measured by familiarity, was left intact, while controlled processing, as measured by recollection, declined.

A.1.2 Aging and Brain Structure

Cognitive aging, as indicated in the brief summary of age-related changes in the preceding section, ultimately is driven by age-related change in the structural properties of the brain. Brain volume decreases as we age, but these declines are not uniform throughout the brain and are due to processes such as cortical thinning and regional volume decline (Hedden & Gabrieli, 2004; N. Raz, 2000; Salthouse, 2011). Although both grey and white matter decline with aging (Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003), volumetric declines in the brain differ by brain region: declines are especially observed in the hippocampus (Lövdén et al., 2012; N. Raz, Ghisletta, Rodrigue, Kennedy, & Lindenberger, 2010; Uylings & de Brabander, 2002), cerebellar cortex, cerebellar white matter, caudate (N. Raz et al., 2013, 2010), entorhinal cortex (N. Raz et al., 2010), frontal lobes (N. Raz et al., 2013; Resnick et al., 2003; Uylings & de Brabander, 2002), and parietal lobes (Resnick et al., 2003).

In addition white matter integrity in the brain deteriorates (Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009; Madden, Bennett, & Song, 2009) as observed in diffusion tensor imaging (DTI) measures of mean diffusivity (MD) and fractional anisotropy (FA) (Madden et al., 2012), as well as axial diffusivity ($\lambda 1$), and radial diffusivity (RD) (Bennett, Madden, Vaidya, Howard, & Howard, 2010; Burzynska et al., 2010; Klawiter et al., 2011).

Individual differences in trajectory of structural changes vary significantly, as seen in both Raz et al. (2010) and Raz et al. (2013). More broadly, many factors in addition to age then are likely to influence both the rate of cognitive change and structural brain change in older adulthood.

A.1.3 Lifestyle Factors

These factors that influence cognitive and structural brain changes in older adulthood include lifestyle factors such as social network, cognitive activities, depression, diet, and exercise. Alterations to all lifestyle factors then can have positive impact on cognitive function.

Social Network

Crooks, Lubben, Petitti, Little, & Chiu (2008) looking longitudinally over 4 years at a large group of elderly women, used the abbreviated Lubben Social Network Scale (LSNS-6; Lubben et al., 2006) to assess social network and the 23-question Telephone Interview for Cognitive Status (TICS-m; Welsh, Breitner, & Magruder-Habib, 1993) to assess cognitive function. They observed that larger social networks appeared to correlate with cognitive status, and concluded that larger social networks had a protective influence on cognitive function. Seeman, Lusignolo, Albert, & Berkman (2001) looked longitudinally over 7.5 years in the high-functioning (top third physically and cognitively) subsample of the MacArthur Studies of Successful Aging (Berkman et al., 1993). Controlling for baseline cognitive function and other socioeconomic, behavioral, psychological, and health status predictors of cognitive function, the authors found that more emotional support was a significant predictor of better follow-up cognitive function, estimated from measures of language, abstraction, spatial ability, delayed spatial recognition, incidental recall, and delayed recall. Emotional support was measured by questionnaire and based on average frequency of emotional support from spouse, children, close friends, and relatives. Perhaps then additional emotional support is the reason that those with larger social networks display less decline in cognitive function. In addition, larger and more complex social networks are also related to greater amygdala volume (Bickart, Wright,

Dautoff, Dickerson, & Barrett, 2011). In their study, Bickart et al. (2011) used the Social Network Index (SNI; Cohen, Doyle, Skoner, Rabin, & Gwaltney, 1997) and defined network complexity as the number of different groups a participant's contacts belonged to, measured by the Number of Embedded Networks Subscale of the SNI. Brain region volume was assessed using automated segmentation and probabilistic region of interest (ROI) labeling in Freesurfer (http://surfer.nmr.mgh.harvard.edu/). Kanai, Bahrami, Roylance, & Rees (2012) using the SNI in younger adults again observed that social network size correlated with amygdala volume using VBM analysis. Interestingly, the authors also looked at online social network size, which correlated with five of eight real-world social network measures of the Social Network Size Questionnaire (Stileman & Bates, 2007) and they observed that again greater network size was associated with increased volume in the amygdala and also right superior temporal sulcus, left middle temporal gyrus and entorhinal cortex. The additional areas correlated only with online social network size so may be specific only to this network measure, but these areas have also been previously implicated in social perception and associative memory.

Cognitive Activities

There are many studies showing that more frequent cognitive activities in older adults is related to better cognitive function (Christensen & Mackinnon, 1993; Hultsch, Hammer, & Small, 1993; Lachman, Agrigoroaei, Murphy, & Tun, 2010; Wilson et al., 1999) and longitudinally, less cognitive decline (Hultsch, Hertzog, Small, & Dixon, 1999; Wilson et al., 2003). For example, Wilson et al. (2005) looked at frequency of cognitive activities in a large cohort of older adults, using a questionnaire they developed; cognitive function was looked at using a battery of 19 tests in 5 domains: episodic memory, semantic memory, working memory, perceptual speed, and visuospatial ability. The authors observed that more frequent current cognitive activity is associated with better cognitive function, especially semantic memory and perceptual speed. Marquine et al. (2012) later confirmed using the same cohort in addition to

the Minority Aging Research Study (MARS, Arvanitakis, Bennett, Wilson, & Barnes, 2010) cohort that the strength of this relationship was the same in Hispanic, non-Hispanic white, and non-Hispanic black participants. Structurally, level of cognitive activities, measured by the Florida Cognitive Activities Scale (FCAS; Schinka et al., 2005) in a sample of older adults, those with Mild Cognitive Impairment (MCI), and those with early stage Alzheimer's disease, were found to be inversely related to estimates of brain atrophy in medial temporal lobe structures as well as cognitive measures (Schinka et al., 2010).

Because of this evidence of beneficial associations with frequency of cognitive activities in older adults, cognitive interventions were undertaken. Early interventions focused on giving participants mnemonic strategies to improve episodic memory performance; Verhaeghen, Marcoen, & Goossens (1992) in a meta-analysis observed that participants given any type of mnemonic strategy performed better in tasks related to that strategy than those in control and placebo groups. However the authors did not look at tasks unrelated to the given strategy for generalization or transfer effects. Later studies focused on cognitive training, where participants practiced tasks targeting certain domains of cognition; Martin et al. (2011) in a meta-analysis of 36 studies in older adults and people with MCI found no significant benefit of cognitive training when compared to active control groups taking part in other types of training. Also Melby-Lervåg & Hulme (2013) in a meta-analysis of 23 studies using working memory task with participants of all ages, including children with attention-deficit/hyperactivity disorder (ADHD), observed improvement in working memory task performance but no transfer of improvements to other domains. This lack of transfer to other domains reveals then a lack of improvement in processing efficiency from the cognitive training but instead only acquisition of knowledge relevant for the particular type of task trained (Lövdén, Bäckman, Lindenberger, Schaefer, & Schmiedek, 2010). Perhaps interventions focused on providing mnemonic strategies or increasing cognitive activity in laboratory-type tasks in the shorter term are not effective in boosting overall general cognition. This does not

however rule out the impact of long-term cognitive activities on one's cognitive function, especially over one's lifetime; this is corroborated by the fact that in studies measuring cognitive activities longitudinally, the amount of cognitive activities does not change almost at all over the evaluated period (Hultsch et al., 1999; Marquine et al., 2012; Wilson et al., 2003).

Diet

There has been much interest in the potential for Mediterranean diet to influence cognitive aging and to have demonstrable effects on measures of cognition. The Mediterranean diet consists of abundant plant-based foods, olive oil as the main source of fat, low to moderate amounts of dairy, low to moderate amounts of fish, low to moderate amounts of poultry, 2-4 eggs per week, low amount of red meat, and low-to-moderate amounts of wine (Bach-Faig et al., 2011; Féart, Samieri, Allès, & Barberger-Gateau, 2013; Willett et al., 1995). Adherence to Mediterranean-style diet in relation to cognition in older adults has been explored in detail in different areas of the world. For example, Tangney et al. (2011) observing a very large study of older adults enrolled in the ongoing longitudinal Chicago Health and Aging Project (CHAP), tested cognitive function using the Mini Mental State Exam (MMSE; Folstein, Folstein, & McHugh (1975), immediate and delayed recall, and the Symbol Digit Modalities Test (A. Smith, 1984); and quantified adherence to Mediterranean diet in comparison to Greek population intake (Panagiotakos, Pitsavos, Arvaniti, & Stefanadis, 2007). The participants were followed up every 3 years with the analytic sample reflecting 7.6 years of follow-up. The authors observed that higher adherence scores to Mediterranean diet were associated with reduced decline in cognitive function.

However Opie, Ralston, & Walker (2013) in a review of 11 studies, including the Tangney et al. (2011) study, observed that adherence to Mediterranean diet was only associated with higher MMSE scores and less risk for dementia. In other cognitive tasks the relationship was not clear. Féart et al. (2013) proposed though that this lack of clarity may come from differences in

Mediterranean diet adherence assessment methodology, with some using population-specific median of intake as thresholds and therefore not comparable with other populations while others like Tangney et al. (2011) used Greek population intake as their standard; also some of the studies did not control for other lifestyle factors that may affect cognition. Therefore this is an area that still needs further exploration with the same thresholds for adherence regardless of the population sampled while controlling for other lifestyle factors. Adherence to Mediterranean diet may still prove to be related to more than just MMSE change in older adults.

A.1.4 Exercise

The lifestyle factor we were interested in for our study is exercise.

Cognitively, exercise may protect against age-related change. This has been evidenced in many studies including retrospective (Middleton, Barnes, Lui, & Yaffe, 2010), cross-sectional (Benedict et al., 2012; Eskes et al., 2010; Nemati Karimooy, Hosseini, Nemati, & Esmaily, 2011), medium (Weuve et al., 2004) and long-term studies (Barnes, Yaffe, Satariano, & Tager, 2003; Middleton, Mitnitski, Fallah, Kirkland, & Rockwood, 2008), where increased exercise or fitness has been shown to confer cognitive benefits measured mostly using the limited MMSE or one or two cognitive tasks. Systematic reviews of studies involving aerobic exercise interventions, from 2 months to 6 years duration, also concluded that exercise improves cognitive ability, especially executive function, in normal healthy older populations (Angevaren, Aufdemkampe, Verhaar, Aleman, & Vanhees, 2008; Chang, Labban, Gapin, & Etnier, 2012; Colcombe & Kramer, 2003; Etnier et al., 1997; Hindin & Zelinski, 2012; P. J. Smith et al., 2010). Smith et al. (2010) in their meta-analytic review of randomized control trials (RCTs) found that exercise improved attention, processing speed, executive function, and memory in healthy older populations. However in all of these reviews the effect sizes were not large.

Again changes in cognition are ultimately driven by changes in brain structure. Structurally, exercise may induce change in the aging brain or at least change the trajectory of decline (for reviews see Bherer, Erickson, & Liu-Ambrose, 2013; Hayes, Hayes, Cadden, & Verfaellie, 2013). Using both cross-sectional study and a 6-month RCT with sedentary older adults, Colcombe et al. (2003, 2006), observed that the brain regions most affected by aging also showed the greatest benefit of fitness (Colcombe et al., 2003). These areas affected by aging may be the most amenable to changes from exercise. In grey matter this was in the prefrontal, superior parietal, and temporal cortices; in white matter this was in the anterior tracts and transverse tracts running between frontal and posterior parietal lobes (Colcombe et al., 2003). Longitudinally the authors found that aerobic exercise was associated with increases in brain volume in both grey and white matter with the largest increases in areas of the frontal lobes (Colcombe, Erickson, Scalf, Kim, Prakash, Mcauley, et al., 2006). Ruscheweyh et al. (2011) in an intervention with older adults also observed correlations of increase in exercise with increase of the grey matter of prefrontal areas and the cingulate. Erickson et al. (2009, 2011), looked exclusively at the hippocampus in both a cross-sectional study and a 1-year RCT with sedentary older adults and observed that higher levels of aerobic fitness were correlated with greater hippocampal volume and enhanced spatial memory function in older adults (Erickson et al., 2009); and aerobic exercise was associated with increased hippocampal volume instead of decrease, which correlated with improved spatial memory (Erickson et al., 2011). Exercise then may be driving volumetric changes in the brain in both grey and white matter that result in better cognitive performance.

A.1.5 Mechanism

What factors then are driving these exercise-induced reductions in decline in both cognition and brain structure? Just indirectly, increased cardiorespiratory fitness from exercise reduces the risk of medical conditions (i.e. cardiovascular disease, cerebrovascular disease,

hypertension, diabetes) associated with poor cognitive function in older adults (Anstey & Christensen, 2000).

Physiologically, exercise, specifically aerobic exercise, has been shown to produce vascular changes: acutely increasing oxygen saturation, and increasing cerebral blood flow (CBF) in areas related to cognitive function (Lojovich, 2010). CBF meets the metabolic needs of the brain and removes waste (Lojovich, 2010). However with aging, cerebral blood flow progressively declines (Lucas et al., 2012). Lucas et al. (2012) observed in both younger and older adults that greater CBF, quantified using transcranial Doppler, was related to better attentional control in a Stroop task. Long-term exercise, observing in the rat model, has been related to lasting increases in CBF and angiogenesis (Lojovich, 2010). Therefore this increase in CBF and angiogenesis from exercise may result in better cognition.

Aerobic exercise has also been shown to change neurotransmitter concentrations including dopamine and norepinephrine, both of which are involved in cognitive performance (Lojovich, 2010). Also, showing brain plasticity in the rat model, exercise has been observed to up-regulate (stimulate) hippocampal gene expression of neurotransmitter receptors and transporters as well as brain-derived neurotrophic factor (BDNF) (Molteni, Ying, & Gómez-Pinilla, 2002). Most of the up-regulated genes also interact with BDNF, leading the authors to suggest that BDNF has a central role in the effect of exercise on brain plasticity.

This brain plasticity from exercise may be driving better cognitive plasticity, where processing speed increases or representations, like knowledge of a task, are altered resulting in better performance on cognitive tasks (Lövdén et al., 2010). Indeed BDNF is implicated in neurogenesis, synaptogenesis, dendritic branching, and neuroprotection (Lojovich, 2010; Lu & Gottschalk, 2000). Increases in BDNF have been shown to increase from exercise in humans as well (Ferris, Williams, & Shen, 2007) and BDNF levels have been observed to positively correlate with certain measures of cognitive function, including MMSE, word list recognition

and recall, and a modified Boston Naming test, in older women (Komulainen et al., 2008). Therefore increase in BDNF due to exercise may also improve cognitive function.

Exercise is also related to improvements in mood and depression (Rimer et al., 2012). Depressive symptoms were observed to be related to cross-sectional cognitive impairment in a large epidemiological study (Ganguli, Du, Dodge, Ratcliff, & Chang, 2006) and in a meta-analysis of 69 studies, depression severity was correlated with performance in episodic memory, executive function, and processing speed (McDermott & Ebmeier, 2009). Structurally, in a meta-analysis of 12 studies, depression was related to less volume in the hippocampus (Videbech & Ravnkilde, 2004). Therefore improvements in mood from exercise may also mediate the positive effects of exercise on cognition.

If these are some of the mechanisms for increases in cognitive function from at least acute exercise, then are the benefits from these mechanisms sustained in longer-term exercise regimes?

A.2 My Thesis

My thesis focuses on the effects on cognitive function and brain structure of long-term high-effort endurance exercise in older adults. This is an area that other recent studies have started exploring as well. Winker et al. (2010) looking at older athletes against a sedentary control group, observed that out of a battery of cognitive tests, athletes only performed better in non-verbal fluency and trended as performing better in a Stroop task. Also Tseng, Uh, et al. (2013), comparing older athletes to sedentary older adults, observed that older athletes performed better on the executive function tasks of category fluency and letter fluency. Also in terms of brain structure, the older athletes had more grey matter volume in right parietal lobe, cuneus, and the culmen of the cerebellum and more white matter concentrations in precuneus, subgyral occipital lobe, and inferior temporal subgyral temporal lobe. In similar cohorts using different structural scans, Tseng, Gundapuneedi, et al. (2013) reported their athletes had

better white matter integrity in brain regions associated with motor function, visuospatial function, motor control and coordination, and memory function. The cross-sectional designs of these studies though, meant that there was no way to unequivocally attribute the observed differences to the exercise regimen of the volunteers.

Indeed, many of the aforementioned behavioral and MRI cross-sectional studies compared groups that were not expressly similar on lifestyle factors - including cognitive activities, social network, depression, and diet - all of which complicate interpretation of the data. In addition, most of the studies either were interventions with sedentary volunteers or used sedentary volunteers as their control group. Studies comparing groups similar in all key lifestyle measures and with non-sedentary control groups are necessary to get a clearer picture of the long-term effects of exercise.

This PhD thesis engaged two older adult populations that were distinctly different in exercise profile. The first group, super veteran athletes (“supervets”), was engaging in long-term (minimum 20 years) high-effort endurance exercise. The second group, non-sedentary control volunteers, was similar in age, sex, and full-time education to the supervet group, but not exercising beyond regular levels. With these populations we set out to test the effects of long-term high-effort endurance exercise on cognitive performance and brain structure in older adults. Our groups were similar in age, sex, education, intelligence, depression level, social network, cognitive activities, adherence to Mediterranean diet, and potential for physical fitness. More importantly, we report longitudinal measures of performance and brain structure over a 12-month period, so that in our study we can establish not only whether there are differences between the groups but also whether age-related changes over 12 months differentiate the groups.

The following studies are derived from a single cohort that were tested at baseline and 1-year follow-up behaviorally, while a subset of the participants in parallel took part in baseline and 1-year follow-up structural MRI scans.

A.3 My Papers

A.3.1 Article I

In my first paper, I focused on memory, specifically prospective memory. I particularly focused on prospective memory since the effect of exercise on prospective memory has not been reported yet to my knowledge. I included both focal and non-focal conditions in the prospective memory tasks because these conditions theoretically utilize different cognitive processes for optimal performance: focal measuring recovery of the prospective intention by relatively automatic processing and non-focal requiring monitoring and therefore the investment of attentional processing resources (McDaniel & Einstein, 2000).

Exercise may affect these processes differently. There are also individual differences in performance; some of this has been explained by perceived importance of the task to the participant (Kliegel, Martin, McDaniel, & Einstein, 2004) and personality differences (Cuttler & Graf, 2007; Salthouse, Berish, & Siedlecki, 2004). I also included measures of speed of processing and executive function using standard neuropsychological tasks. This was so that I could observe if any differences on memory tasks were related to differences in speed of processing and/or executive function.

I had two predictions on performance on PM tasks that depended on how benefits from long-term high-effort endurance exercise manifested. If the exercise has conferred less decline in processing resources, then in conditions that require more processing, like in the monitoring required for non-focal PM conditions (McDaniel, Shelton, Breneiser, Moynan, & Balota, 2011), there would be a performance advantage for supervets. If the exercise has conferred less

decline in processing speed, like in the automatic processing usually utilized in in the focal PM conditions (McDaniel et al., 2011), there would be a performance advantage for supervets. I also took measures of episodic memory, incidental memory and working memory, which I hypothesized would show benefits of long-term high-effort endurance exercise, since previous literature on memory and exercise has observed a positive association.

A.3.2 Article II

In my second paper, I focused on attention, specifically visual search, sustained attention, and attentional control tasks; covering the three main attentional aspects: selectivity, intensity, and executive attention respectively (McAvinue et al., 2012; Parasuraman, 1998; A. Raz & Buhle, 2006). Previously, in sustained attention tasks, as processing demands increase, the deficit of older adults when compared to younger adults becomes larger (Deaton & Parasuraman, 1993). Interestingly, when accounting for differences in age-related processing demands then, more intrinsic motivation seemed to result in better task performance instead of age differences (Tomporowski & Tinsley, 1996). Related to this, higher interest in sustained attention tasks have also resulted in better performance (Deaton & Parasuraman, 1993); less interest in a task then may preclude the optimal utilization of attentional resources for that task.

In attentional control tasks, such as suppressing attention and switching attention, older adults have been show to perform worse than younger adults (Coubard et al., 2011). Also between-participant variability was observed to increase with age (Coubard et al., 2011). Perhaps differences in exercise regime and physical fitness account for some of this variability.

Indeed Winker et al. (2010) in their study of athletes and sedentary controls saw a trend in better performance on the Stroop attentional control task in interference trials in the athletes. Also recently in a modified to be more difficult Stroop task, higher physical fitness was associated with better performance in the most challenging condition, seemingly reflecting an

increase in attentional control (Prakash et al., 2011). However, two meta-analyses of exercise intervention RCTS that included standard Stroop tasks as a measure, observed no differences in this task from the exercise interventions (Angevaren et al., 2008; P. J. Smith et al., 2010). More recently, in an exercise RCT that modified the Stroop task to be more difficult, again only in most difficult condition did the exercise improve performance (Predovan, Fraser, Renaud, & Bherer, 2012). Therefore in my study, I included a more difficult Stroop task that included a switching component (Hutchison, Balota, & Ducheck, 2010).

In addition, higher physical fitness has been observed to attenuate the age-related performance decline in sustained attention tasks as well (Bunce, Barrowclough, & Morris, 1996; Bunce, 2001).

For this paper, I predicted that the attenuation of age-related decline in attentional capabilities from long-term high-effort endurance exercise would give benefit to attention task performance for supervets, and this would be particularly pronounced at least in the more difficult Stroop-switch task.

A.3.3 Article III

In my third paper, I focused on structural changes in the brain, using anatomical and DTI scans. The anatomical scans allowed us to look at brain tissue volume and changes in these volumes. The DTI scans allowed us to look at white matter integrity. I also explored whether there were relationships between any significant or trending differences between supervets and controls in cognitive tasks and structural MRI measures.

I used similar or improved methods used in previous papers exploring aging or aging and exercise. These methods included whole volume analysis (Erickson et al., 2009, 2011; Resnick et al., 2003), automated segmentation (Resnick et al., 2003), Voxel-Based Morphometry (VBM) (Tseng, Uh, et al., 2013), longitudinal VBM (Colcombe et al., 2003; Colcombe, Erickson, Scalf,

Kim, Prakash, McAuley, et al., 2006), and Tract-Based Spatial Statistics (TBSS) (Tseng, Gundapuneedi, et al., 2013).

Raz et al. (2013, 2010) used manual tracing to segment their volumes, but automated segmentation has been shown to be highly correlated in volume and shape with manual tracing especially using Freesurfer (Morey, Petty, & Xu, 2009) with the additional benefits of no operator bias and taking less operator time. For volume and cortical thickness analyses we looked specifically at areas most previously seen to be affected by aging and/or were previously shown to be different for those that exercised. For volumes we chose hippocampus (Erickson et al., 2009, 2011; Lövdén et al., 2012; N. Raz et al., 2010; Uylings & de Brabander, 2002), cerebellar cortex (N. Raz et al., 2013, 2010; Tseng, Uh, et al., 2013), cerebellar white matter (N. Raz et al., 2013, 2010; Tseng, Uh, et al., 2013), and caudate (N. Raz et al., 2013, 2010). For cortical thickness we looked at entorhinal cortex (N. Raz et al., 2010), frontal lobes (Colcombe et al., 2003; Colcombe, Erickson, Scalf, Kim, Prakash, McAuley, et al., 2006; N. Raz et al., 2013; Resnick et al., 2003; Uylings & de Brabander, 2002), and parietal lobes (Colcombe et al., 2003; Resnick et al., 2003; Tseng, Uh, et al., 2013).

With my DTI measures, I also used the more recently developed histogram analysis, which is sensitive to subtle diffuse differences in the brain (Tofts, Davies, & Dehmeshki, 2003), whereas TBSS detects localized differences (S. M. Smith et al., 2006).

I predicted that long-term high-effort endurance exercise would result in supervets having greater brain volume than controls, especially in the hippocampus. I also predicted that supervets would have better white matter integrity than controls.

A.4 Remarks

My final discussion provides an overview of the findings from the three papers, potential explanations for the findings, study limitations, and potential future directions for this research.

B. **Article I:** Cognitive Effects of Long-Term High-Effort Endurance Exercise in Older Adults: Are There Measurable Benefits to Memory?

Abstract

Age-related decline in cognitive performance can be offset by increased exercise. Little is known, however, about the cognitive consequences of long-term high-effort endurance exercise, especially in comparison to non-sedentary lifestyles. In a cross-sectional design, we recruited older adults who had been engaging in high-effort endurance exercise over at least twenty years, and compared their cognitive performance with a non-sedentary control group similar in age, sex, education, IQ, depression levels, and other lifestyle factors. We hypothesized that long-term high-effort endurance exercise would protect against the age-related decline in executive function and memory including prospective memory, an area that remains underexplored in relation to the effects of exercise. Our findings, in contrast to previous studies, indicated that those participating in long-term high-effort endurance exercise, when compared without any confounds to non-sedentary control volunteers, showed no differences on measures of speed of processing, executive function, incidental memory, episodic memory, and working memory. On measures of prospective memory, long-term exercisers performance suggested a self-imposed increase in effort, which did not impact on ability to complete the PM task.

Article I has been submitted to PLOS ONE.

B.1 Introduction

Considerable interest is growing in the potential of exercise to protect against age-related change in cognition. Many studies have tried to quantify this in specific domains using different experimental techniques, and in retrospective and short to medium term studies, where increased exercise has been shown to confer cognitive benefits (Angevaren et al., 2008; Chang et al., 2012; Colcombe & Kramer, 2003; Etnier et al., 1997; Hindin & Zelinski, 2012; P. J. Smith et al., 2010).

For example, Middleton, Barnes, Lui, & Yaffe (2010) conducted a retrospective study of 9344 older women, using self-reports of physical activity of up to 50+ years in the past, and suggested that older women (ages 65 and above) who reported being physically active at any point over their life course, had a lower likelihood of cognitive impairment (as measured by modified MMSE) in later life.

Longitudinal studies looking at more specific domains also confirmed exercise-associated benefits. Weuve et al. (2004) in a 2-year large group longitudinal study of self-reported physical activity in older female nurses (ages 70-81) and Barnes, Yaffe, Satariano, & Tager (2003) in a 6-year large group longitudinal study measuring cardiovascular fitness in older adults (ages 55 and above) both reported better cognitive performance and less cognitive decline over time in physically active individuals.

Systematic reviews of studies involving short-term aerobic exercise interventions, from 2 months to 6 years duration, also concluded that exercise improves cognitive ability, especially executive function, in normal healthy older populations (Angevaren et al., 2008; Chang et al., 2012; Colcombe & Kramer, 2003; Etnier et al., 1997; Hindin & Zelinski, 2012). Smith et al. (2010) in their meta-analytic review of RCTs of limited duration interventions found that exercise improved attention and processing speed, executive function, and memory in both healthy populations and in people with mild cognitive impairment. Brown et al. (2012), analyzing

intensity peaks in daily exercise profiles of the Australian AIBL older adult cohort, concluded that exercise intensity modulated cognitive performance, though differences in favor of higher intensity exercisers emerged only on a proportion of the executive function measures that they took.

Linking behavioral findings with functional connectivity in the brain, Voss et al. (2010) compared healthy young adults (ages 18-35) to two older adult (ages 55-80) groups: one group that did flexibility, toning, and balance (FTB) training and another group that undertook a walking regime. They found that moderate exercise over 1 year enhances functional connectivity between regions normally exhibiting age-related disruption, specifically the Default Mode Network and the Frontal Executive Network, the latter associated with greater improvement in a composite measure of executive function. The connectivity enhancement was apparent in both FTB training and walking interventions but the effect was more pronounced for the walking group.

Brain structure is also modulated by exercise, even in older adults. Erickson et al. (2011) showed that a 1-year aerobic exercise intervention increased hippocampal volume in older adults (ages 55-80), while hippocampal volume decreased in volunteers in their stretching control group. Greater increases in fitness were correlated with greater increases in hippocampal volume, and there was a positive relationship between higher aerobic fitness levels and spatial memory and between increased hippocampal volume and improved spatial memory. Also the study interestingly found that while hippocampal volume declined in the stretching group overall, higher fitness level before the intervention was still protective against volume loss and partially attenuated the decline in volume.

Exploring cerebral perfusion and executive function at rest and during exercise itself in both younger and older adults, Lucas et al. (2012) reported that executive function improved during exercise and middle cerebral artery blood flow velocity at rest was strongly related to cognitive

performance. They hypothesized that regular exercise, which improves cerebral perfusion, can improve or maintain cognitive performance.

Two very recent studies have begun to explore the effects of more extreme exercise regimens in older adults. Winker et al. (2010) in the Austrian APSOEM trial recruited elderly (ages 60 and above) runners or bicyclists, who had participated in at least one marathon in the previous two years, and an inactive control group similar in age, sex, and education. The cognitive tests they used included measures of visuo-construction, attention, verbal and figural memory and the executive functions of planning, shifting and task switching, nonverbal fluency, verbal fluency, and interference. Athletes performed better than the controls only in the measure of nonverbal fluency, although a trend was noted in the Stroop Test. Both were measures of executive function. They suggested that fitness may not produce cognitive benefits across all domains, but that, in line with Colcombe & Kramer's (2003) meta-analysis finding greater positive effect sizes of exercise in executive tasks, executive function may be most sensitive to benefits.

Most recently, Tseng, Uh, et al. (2013) reported a study that compared a small group of twelve older runners with sedentary older adults on a battery of cognitive tasks focusing on executive function and memory. After controlling for IQ difference between their groups, their runners performed better than their sedentary older adults only in the executive function tasks of category fluency and letter fluency. They suggested, again, that exercise training selectively benefits executive function in older adults. VBM measures of brain volume indicated that their older athletes had more grey matter and white matter concentrations in right parietal lobe than sedentary older adults and these were correlated with aerobic fitness levels in their athletes. They hypothesized that these areas had been preserved because of visuospatial and motor stimulation during exercise. Also, since these areas did not completely overlap with age-related tissue concentrations differences, they suggested that exercise may also preserve brain

tissue concentrations in regions not influenced by aging. In the same cohort, Tseng, Gundapuneedi, et al. (2013) reported better white matter integrity, as indexed by higher fractional anisotropy and lower mean diffusivity in regions of the brain associated with motor and memory function and lower deep white matter hyperintensity volume, suggesting that long-term exercise may also preserve white matter integrity from age-related changes.

Many of the aforementioned studies either were interventions with sedentary volunteers or used sedentary volunteers as their control group. Also many of the cross-sectional studies compared groups that were different on measures of lifestyle, age, gender, and depression, all of which complicate interpretation of the data. In addition whether there is a positive linear relationship between exercise and cognitive benefit is yet to be explored: is there a ceiling to the benefit or does more exercise always mean better cognition?

In addition one particular domain that has not yet featured in the assessments of exercise is prospective memory. Prospective memory (PM) is the memory for future intentions: intentions that cannot be realized immediately after their formation, but must be retained and recalled at a timely moment in the future (Kvavilashvili & Ellis, 1996). PM is the memory we employ in a multitude of everyday tasks, and is particularly relevant to sustained independent living as we grow older. Time-based prospective memory occurs when one performs a specific behavior at a pre-specified time, for example remembering to call a friend at a designated later time. Event-based prospective memory, on the other hand, occurs when one performs a specific behavior when prompted by an external cue, for example remembering to buy milk at the grocery store the next time you pass by (Einstein & McDaniel, 1990).

McDaniel & Einstein (2000) in their multiprocess framework suggested that event-based prospective memory can be supported either by monitoring the environment for a prospective cue (which absorbs attentional resources) or by relying on the prospective cue to automatically prompt the intended action. Whether one relies on monitoring or automatic processes

depends on the characteristics of the ongoing task, the specific PM task, and the individual. According to McDaniel & Einstein (2000), if the ongoing task already requires processing of the defining features of the prospective memory cue (described as a focal PM task), then automatic processes are sufficient to activate the PM intention. In tasks in which the prospective memory cues are not part of the information being extracted in the ongoing task (described as a non-focal PM task), then monitoring for the cue is required.

McDaniel & Einstein (2000) also suggested that that the perceived importance of the PM task modulates whether one uses monitoring or more automatic processes in prospective remembering. Kliegel, Martin, McDaniel, & Einstein's (2004) reported that the importance of the PM task indeed increased attentional costs (an index of monitoring) and also improved PM performance if the PM task itself required monitoring. Conversely, they found no improvement in PM performance if the "important" PM task did not require the additional monitoring.

Smith & Bayen (2004) differed from McDaniel & Einstein in suggesting that there is always a cost when carrying a PM intention. They suggested, in their preparatory attentional and memory (PAM) theory, that non-automatic preparatory attentional processes must be engaged to perform any PM component of a task, which then reduces the resources available to the ongoing task. The preparatory attentional processes are shared with the ongoing task as well, so better PM performance may be due to increased monitoring which occurs at a greater cost to performance on the ongoing activity.

Smith (2003) demonstrated evidence to support PAM theory when analyzing performance of the ongoing task independent of PM target trials and trials immediately preceding or following PM target trials. In this way, any cost found could not be associated with just performing the action associated with the PM intention. She reported longer reaction times in the ongoing task when participants were carrying a PM intention and that the longer reaction times were

associated with better prospective memory. She concluded that there was always a cost associated with having an embedded PM intention.

McDaniel & Einstein (2000) also indicated that individual differences can affect prospective memory performance under certain conditions. Some personality characteristics have previously been linked to higher PM performance, and they suggest that this may be because of a tendency for certain individuals to implement increased monitoring. Cuttler & Graf (2007) sought to bring evidence to this and in their study used a test battery that included two naturalistic laboratory PM tasks and one naturalistic field PM task. In addition they used questionnaires measuring the Big 5 aspects of personality and perfectionism. They found that conscientiousness was positively correlated with their field task and one of their laboratory tasks. Neuroticism was also positively correlated to the same laboratory task. Socially described perfectionism though was negatively correlated with performance on the other laboratory task.

In contrast to this, Salthouse, Berish, & Siedlecki (2004) only found one positive correlation between the Big 5 personality dimensions and PM performance in their laboratory computer-based PM task: agreeableness. This suggests a difference may exist in the contribution of personality characteristics to naturalistic vs. laboratory computer-based PM tasks. It also informs us that individual differences in personality may indeed affect PM performance and further exploration in this area is needed.

Age is also a factor that affects PM performance. Henry, MacLeod, Phillips, & Crawford's (2004) meta-analysis reported large age-related deficits in prospective memory, with the most substantive differences emerging in laboratory-based measures of PM, and in tasks where the PM cues were non-focal, thus engaging cognitive resources. Following on, Rose, Rendell, McDaniel, Aberle, & Kliegel (2010) using a “virtual week” computerized board game set out to look at age differences in PM with a paradigm designed to tap more naturalistic application of

PM. They reported that the attentional resources engaged in the completion of PM tasks were age sensitive and that working memory was correlated with PM performance in non-focal cue conditions, but this correlation was reduced to non-significance for PM tasks with regular or focal cues.

Also exploring cue focality, the meta-analysis of Kliegel, Jäger, & Phillips (2008) found that age-related declines are larger for non-focal PM than for focal PM tasks. According to McDaniel and Einstein's (2000) multiprocess theory, this would make sense if aging affected higher order processing which is required for the monitoring used in non-focal PM tasks. According to multiprocess theory, the focal PM task should not see a decline at all since it involves automatic processes. This position is somewhat at odds with the outcome of the meta-analysis; however a weaker prediction for the multiprocess theory remains: even focal PM tasks showed an age-related decline, although to a lesser extent than non-focal PM tasks. Alternatively, the result could also be accommodated by Smith & Bayen's (2004) preparatory attention and memory (PAM) model, which argues that there is always a cost when performing a PM task: aging may just be increasing that cost to a level that is statistically significant.

In summary, the prevailing evidence suggests that at least the non-focal PM task is likely to engage significant cognitive resource, and therefore be susceptible to age-related deficits as cognitive resources diminish with age. In addition, strategic prioritizing of the tasks, through instruction bias, or as a result of personality factors, may also elicit performance differences when resources are limited. For these reasons, one might expect that PM performance will be sensitive to exercise-related effects on cognition. Specifically, one might anticipate that beneficial effects of exercise will be most prominent on tasks involving non-focal PM cues, which engage resources for monitoring, while exercise would not differentiate performance on focal PM task, because additional resources would not help in the performance of this type of PM condition.

An alternative prediction arises, however, from a recent study by McDaniel, Shelton, Breneiser, Moynan, & Balota (2011). The authors looked for differences in focal and non-focal PM task performance in healthy older adults and those with very mild dementia (CDR 0.5). While there was no significant difference in the ongoing task reaction times between the no PM and the focal PM conditions, the non-focal PM condition was significantly slower than the no PM condition. This indicated that participants were monitoring for PM cues in the non-focal PM condition, while not doing so in the focal PM condition, consistent with McDaniel & Einstein's (2000) multiprocess framework. They also found that the very mildly demented participants were significantly more impaired on the focal PM condition than healthy controls, while there was no significant difference between the groups in non-focal PM accuracy. This was inconsistent with the notion that only the non-focal task engages resources for monitoring. McDaniel et al. (2011) theorized that the focal PM task differentiated the groups because spontaneous retrieval, used in focal PM tasks, is dependent upon medial temporal structures (Moscovitch, 1992, as cited in McDaniel et al. 2011), including the hippocampus, which are compromised in early Alzheimer's disease (Buckner, 2004, as cited in McDaniel et al. 2011). Following the evidence that positive exercise effects are observed on hippocampal volume in healthy individuals (Erickson et al., 2011), this would lead to the prediction that it is the spontaneous processing, focal PM condition that will differentiate an exercise from a non-exercise group.

The current study engaged two older adult populations that were distinctly different in their exercise profiles. The first group, super veteran athletes ("supervets"), comprised individuals whose age qualified them for super veteran categorization by UK Athletics, and who had engaged in long-term (minimum 20 years) high-effort endurance exercise. The second group, non-sedentary control volunteers, was similar in age and full-time education to the supervet group, socially active, but not exercising beyond regular levels. With these populations we set out to test the effects of exercise on cognitive performance in older adults, and in particular

whether measures of PM would be sensitive to differences in exercise regime, and might differentiate according to the alternative hypotheses proposed by McDaniel et al. (2011). Participants were tested at two time points, 1 year apart, so that we would also be able to ascertain whether age-related changes in performance over a 12-month period might differentiate our groups.

B.2 Methods

B.2.1 Ethics Statement

Ethical approval was obtained from the University of Sussex Life Sciences & Psychology Cluster based Research Ethics Committee. Written consent was obtained from all participants.

B.2.2 Participants

For inclusion in the study, we chose non-smoker (never smoked or not smoked in the past 5 years) participants aged 60-85, on stable medication if any during the past 12 months, with English proficiency equivalent to that of a native speaker.

For our supervet group we required active exercise at a high level mostly via running, swimming, and/or cycling (self-paced sports) for the past 20 or more years. We chose only self-paced sports because athletes of interceptive-dominant sports, which usually involves a part of the body or a stick or racquet to interact with a ball, have been shown to have faster reaction times (Voss et al., 2010b) which alone could have an effect on cognitive performance. Also we chose high-effort endurance exercise to differentiate from exercise consisting of just short high-effort bursts or sustained low effort.

We excluded participants if they had a history of stroke, myocardial infarction, recently diagnosed diabetes, very high blood pressure (systolic above 200 and diastolic above 100), psychiatric or neurological disorders (self-reported), or were suffering from clinical depression.

We used the Mini Mental State Exam (MMSE; Folstein, Folstein, & McHugh, 1975), which assesses general cognitive function to exclude for Mild Cognitive Impairment and Dementia. In addition we used the Geriatric Depression Scale (GDS; Sheikh & Yesavage, 1986), which rates symptoms of depression, to exclude for depressed individuals.

We recruited 27 supervets mainly from running and triathlon clubs, but also from park runs, the finish line of races, running shops, as well as word of mouth. We recruited 23 control volunteers from churches, old age associations, women's clubs, university alumni associations, and word of mouth. This was designed to ensure a match for the social interaction that many of the supervets obtained from their participation in the running/triathlon clubs.

We excluded from analysis those diagnosed with a memory disorder at follow-up, resulting in one control volunteer being excluded, leaving 22 control volunteers.

Participants were tested at the University of Sussex campus and were compensated for their transportation costs and parking.

B.2.3 Demographics

Physical Activity

For measuring physical activity/exercise we used the validated Physical Activity Scale for the Elderly (PASE; Washburn, Smith, Jette, & Janney, 1993).

Diet

To assess diet, we used the EPIC-Norfolk Food Frequency Questionnaire (FFQ; Bingham et al., 2001) and extracted an adherence to Mediterranean diet score. Adherence to Mediterranean diet was scored from 0-9 with each point being based on sex-specific median cut-offs for 9 dietary categories; greater scores represented a greater adherence (Cade, Taylor, Burley, & Greenwood, 2011; Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003).

Physiological

We took lung function measures, using a spirometer (Microplus, Micro Medical Limited, Kent, UK). Lung measures included: Forced Expiratory Volume 1st Second (FEV1), the amount of air expelled from the lungs during the first second after a full inhalation; Forced Vital Capacity

(FVC), the total amount of air expelled from the lungs after a full inhalation; Forced Expiratory Ratio (FER), the ratio between FEV1 and FVC; and Peak Expiratory Flow (PEF), which is the maximal velocity of air blown during the same breath.

We performed a Bioelectrical Impedance Assay (Bodystat Quadscan 4000 or Bodystat 1500; Douglas, Isle of Man, UK) to obtain percent body fat. We measured hand-grip strength, using a hand dynamometer (Grip-D, Takei Scientific Instruments, Japan).

IQ

We used the National Adult Reading Test (NART; Nelson, 1982) to estimate full-scale pre-morbid IQ.

Social and Cognitive Engagement

We used the 6-question Lubben Social Network Scale (LSNS; Lubben et al., 2006) to assess social relationships in terms of family and friendship ties and the Florida Cognitive Activities Scale (FCAS; Schinka et al., 2005) to assess amount of participation in cognitive activities.

B.2.4 Cognitive Tasks

Speed of Processing & Executive Function

The Digit Symbol Substitution Task (DSST, Wechsler, 1981) measures "motor coordination of speed, learning, visual scanning, as well as perception, visual shifting, and symbol encoding" (Bowler, Sudia, Mergler, Harrison, & Cone, 1992). We included Symbol Copy, which has a speed of processing component, and can be used to isolate higher mental functions in the substitution portion of the task. Subtracting mean time per item in Symbol Copy from the Digit Symbol Substitution yields this index (Glosser et al., 1977; Storandt, 1976 as cited in Joy, Fein, & Kaplan, 2003). We also included the incidental memory portion measuring immediate recall of symbol pairings.

Trail Making Test A and B (Reitan, 1958) is a test of visual conceptual and visuomotor tracking with a speed of processing component in part A and a more complex set switching component in part B. Following convention, we calculated a difference score of "B – A" to remove the speed element from Part B.

Memory

Episodic Memory:

Episodic memory was measured using a 20-item word list presented on the computer screen at the rate of one word every two seconds, followed by an immediate written recall.

Working Memory:

The Backward Digit Span (Wechsler, 1981) was used to assess working memory, and was inserted during the follow-up only. Here participants were given a series of digits and asked to repeat them back backwards to find the maximum number of digits they could repeat back.

Prospective Memory Measures:

Subjective Memory Rating

Prospective and Retrospective Memory Questionnaire (PRMQ; Crawford et al, 2003), a validated self-assessment of a participant's own prospective and retrospective memory.

Event-based PM Tasks

1. Card Sort Task (Rusted, Sawyer, Jones, Trawley, & Marchant, 2009)

Participants pressed corresponding buttons to sort playing cards into hearts and spades, while not responding to diamonds and clubs. After practicing, the first portion consisted of a baseline condition, where participants completed just the ongoing card sort task. Then participants were given a PM intention: to press the spacebar for number 7 cards regardless of

suit. This embedded PM cue, a 7 card, was non-focal since processing the card number was not necessary for the ongoing sort task.

Following a filled interval of approximately 5 minutes of unrelated measures (NART, SRT), participants completed the card sort, with the embedded PM intention, over two complete decks.

2. Focal and Non-Focal Prospective Memory Task (McDaniel et al., 2011)

Here the ongoing task was a category decision task. Participants decided whether a word on the left side of the screen fitted into a category on the right side of the screen, by pressing a "Y" or "N" button for "Yes" and "No" respectively. The ongoing task pairs were counterbalanced to have half "Yes" correct answers and half "No" correct answers. Participants practiced the ongoing category decision task first, which included six trials giving speed and accuracy feedback to encourage optimization of both.

In addition to the ongoing task there were three latin-squares order-counterbalanced prospective memory conditions: 1. A focal PM condition - where participants pressed the "Q" key if they saw one specific word, i.e. Aluminum, Tortoise, or Raspberry; 2. A non-focal PM condition - where participants pressed the "Q" key if they saw a specific part of a word, i.e. "min" as found in words like alu**min**um, "tor" as found in words like his**tor**y, and "ras" as found in words like **ras**pberry; and 3. A no PM control condition. PM targets for the focal and non-focal conditions were presented 3 times during their appropriate condition and not presented again in the other conditions. Volunteers completed an approximately five minute filled interval of unrelated tasks between instruction and test for each condition (Controlled Oral Word Association Task, Trails, DSST).

B.2.4 Procedure

Participants completed a 3-hour session of cognitive and physiological testing in the following order: 10 minutes greet and consent, 15 minutes physiological measures, 75 minutes cognitive measures, 20 minutes tea break, 30 minutes cognitive measures, and 30 minutes questionnaires. Only a subset of the cognitive measures is reported in this paper.

B.2.5 Preliminary review of data, data exclusions

Card Sort Task

Several participants did not remember the PM component of the task or did it wrongly (only pressed for subset of 7s or thought pressing the 7s would occur later on). These participants' data were excluded from the PM condition. One participant was excluded because they were not familiar with playing cards. At the initial time point, these removals constituted 5 datasets out of 54 for the supervets and 4 datasets out of 46 for controls. For one of the supervets due to a computer malfunction no data was recorded for the PM condition of the task, but their no PM condition results were included in the analysis. At follow-up the removals constituted 1 dataset out of a possible 44 in supervets and no datasets for controls.

McDaniel PM Task

Several participants did not complete the focal PM, non-focal PM, or both PM components of the tasks correctly, either because they forgot about the PM intention completely or because they thought the PM component would come in a later condition when prompted. At the initial time point, this constituted 6 data sets from a total of 81 possible datasets for the supervets, and 6 datasets from a total of 66 possible datasets for controls. Also for one control, no data was recorded on the task due to a computer malfunction. At follow-up, this constituted 4 datasets from a total of 66 possible datasets for supervets, and 2 datasets from a total of 54 possible datasets for controls. For one control, no data was recorded on the task due to a computer malfunction.

B.2.6 Statistical Analysis

Demographics

For all statistical tests an alpha of .05 was adopted. To compare means of measures just between groups (supervets vs. controls) at each time point we used independent t-tests. Transforms were used when samples violated normality and/or homogeneity of variance for significances; if transforms had no effect then 1000-sample bootstrapping with bias corrected accelerated confidence intervals were used, as noted. When including both time points in follow-up analysis, mixed ANOVAs were used with group as the between-subject factor and time point as the within-subject factor. Transforms were used when samples violated normality and/or homogeneity of variance for significances; if transforms had no effect then robust mixed ANOVAs using trimmed means were employed, as noted.

Experimental Tasks

For all statistical tests an alpha of .05 was adopted. Transforms were used when samples violated normality and/or homogeneity of variance for significances; if transforms had no effect then 1000-sample bootstrapping with bias accelerated confidence intervals were used, as noted.

Independent t-tests between groups were used to compare means of measures where appropriate, unless otherwise noted. Mixed factor ANOVAs were used to analyze the McDaniel PM task.

Follow-up analyses used mixed factor ANOVAs with time point as a repeated measures factor.

B.3 Results

B.3.1 Demographics

Characteristics

Results for Characteristics are shown in Table B.1.

There were no significant differences between group at the initial time point on any of the measures taken, and no significant changes at follow up (one year later).

Table B.1

Means, Standard Deviations, and Significances Between Supervet and Control Group Characteristics

Measure	Supervets Mean (SD)	Controls Mean (SD)	Significance at T0	Supervets Mean (SD)	Controls Mean (SD)	Significance at T1
Characteristics:						
Sex[a]	6 female	9 female	$p = .158$	6 female	7 female	$p = .577$
Age (years)	67.88 (5.45)	68.35 (5.81)	$p = .771$	69.29 (5.07)	69.21 (5.83)	$p = .965$
Education (years)[b]	15.26 (3.59)	16.18 (3.38)	$p = .363$‡	16.00 (3.59)	16.16 (3.45)	$p = .888$
IQ[c]	117.66 (3.92)	119.93 (4.51)	$p = .077$‡	118.37 (5.72)	120.70 (4.34)	$p = .170$‡
Depression[d]	0.52 (0.89)	1.18 (1.71)	$p = .139$‡	0.71 (1.10)	1.11 (1.82)	$p = .428$‡

Note: a. Chi-squared test used. b. Years of full-time education. c. Full Scale Pre-morbid Intelligence Quotient derived from National Adult Reading Test. d. From Geriatric Depression Scale score. ‡ - From 1000-sample bootstrap results.

Physical Activity

Results for Physical Activity are in Table B.2: Physical Activity.

As expected and desired, on the PASE the supervets took part in more physical activities than the controls at the initial time point and this difference was maintained when including both the initial time point and follow-up in analysis. There was no main effect of time point and no group by time point interaction effect, Qs < 1.

Interrogating the PASE further, at the initial time point supervets took part in significantly more strenuous sports, $t(34.58) = 4.59$, $p < .001$, and muscle strength and endurance activities, $t(30.41) = 2.78$, $p = .009$. When including the follow-up time point in the analysis, there was a group by leisure activity interaction, $F(3.09, 117.33) = 6.20$, $p = .001$. Again supervets ($M = 1.08$

hours per day) participated in more strenuous sports than controls (M = .20), $F(1, 38) = 27.93$, $p < .001$. And again supervets (M = .12) participated in more muscle strength and endurance activities than controls (M = .03), $F(1, 38) = 8.88$, $p = .005$. The groups did not differ in time spent on any other type of leisure activity. For both groups, there was an increase in moderate activity at follow-up (M = .28 hours per day) vs. initial time point (M = .12), $F(1, 38) = 4.31$, $p = .045$, while other activities did not change.[1]

Lung Function

Results for Lung Function are in Table B.2: Lung Function.

At the initial time point, there were no significant differences between groups in lung function measures: FEV1, FVC, FER, and PEF. Lung function measures are not affected by exercise training (Dempsey, 1986), and we can interpret the comparability of groups as evidence that potential for cardiovascular performance in both our groups was similar, and participants in both groups as a whole were capable of achieving the same level of fitness.

When including the follow-up time point, there was no difference between groups in FEV1, FVC, FER, and PEF.

There was no change between time points for FEV1, $F < 1$, and FER, $Q < 1$. FVC had declined from the initial time point in both groups, $F(1, 30) = 5.62$, $p = .024$, and PEF increased from the initial time point to follow-up, $F(1, 33) = 4.97$, $p = .033$.

Time point did not interact with group: FEV1, $F(1, 33) = 2.77$, $p = .105$; FVC, $F < 1$; FER, $Q < 1$; and PEF, $F(1, 33) = 2.30$, $p = .140$.

Diet

Results for Diet are in Table 2: Diet.

[1] However there were violations of normality and some homogeneity of variance violations, so follow-up ANOVAs for the follow-up time point must be interpreted with caution.

There were no differences between groups in adherence to Mediterranean diet at the initial time point.

Physiological

Results for Physiological Measures are in Table B.2: Physiological.

Because of physiological differences between males and females, these measures were analyzed separately per gender.

In physiological measures, expected differences between our groups were confirmed at the initial time point. Supervets had significantly less percent body fat in both sexes, and male supervets trended as having stronger hand-grip than male controls, while females did not differ.

Including the follow-up time point in analysis, supervets again had less percent body fat for both sexes and male supervets had stronger hand-grip than male controls, while again females did not differ.

There was no difference in time point for hand-grip strength in females, $F(1, 10) < 1$. However, hand-grip strength showed an increasing trend for males overall, $F(1, 19) = 3.63$, $p = .072$. Percentage fat also increased in both groups for both sexes: males, $F(1, 24) = 23.25$, $p < .001$; females, $F(1, 11) = 5.46$, $p = .039$.

There were no group by time point interactions: percentage fat males, $F(1, 24) = 1.57$, $p = .222$, percentage fat females, $F(1, 11) < 1$; hand-grip strength males, $F(1, 19) < 1$, hand-grip strength females, $F(1, 10) – 1.03$, $p = .334$.

Social and Cognitive Activities

Results for Social and Cognitive Activities are in Table B.2: Social and Cognitive Activities.

At the initial time point, there were no significant differences between the groups in LSNS score or FCAS score.

When including the follow-up time point in analyses, again for LSNS score there was no main effect of group, no main effect of time point, $Q < 1$, nor group by time point interaction, $Q = 2.14$, $p = .155$. For FCAS score there was no main effect of group, time point, nor group by time point interaction, $Qs < 1$.

Table B.2

Means, Standard Deviations, and Significances Between Supervet and Control Groups Physiological, Social, and Cognitive Characteristics

Measure	Supervets Mean (SD)	Controls Mean (SD)	Significance at T0	Supervets Mean (SD)	Controls Mean (SD)	Significance with T0 and T1
Physical Activity:						
PASE[e]	221.70 (71.24)	169.39 (66.03)	$p = .011$*	230.60 (60.39)	160.87 (55.12)	$p = .001^{\Omega}$*
Diet:						
Mediterranean Diet Score	4.81 (1.62)	4.64 (1.81)	$p = .690^{\ddagger}$			
Lung Function:						
Forced Expiratory Volume 1st Second (L)	2.87 (0.72)	2.78 (0.78)	$p = .686$	2.97 (0.66)	2.86 (0.72)	$p = .999$
Forced Vital Capacity (L)	3.78 (0.87)	3.38 (0.93)	$p = .153$	3.77 (0.84)	3.36 (0.81)	$p = .235$
Forced Expiratory Ratio (%)	78.01 (8.93)	83.20 (8.88)	$p = .079^{\ddagger}$	77.25 (8.92)	84.75 (9.20)	$p = .085^{\Omega}$
Peak Expiratory Flow (L/min)	411.89 (143.83)	421.95 (155.24)	$p = .875$	481.37 (119.32)	458.25 (132.21)	$p = .963$
Physiological:						
Body Fat Males (%)	22.05 (4.07)	26.77 (4.07)	$p = .003$*	25.03 (4.38)	28.14 (4.09)	$p = .031$*
Body Fat Females (%)	32.50 (4.19)	39.38 (6.59)	$p = .042$*	35.45 (2.94)	40.84 (3.44)	$p = .030$*
Hand-grip Strength Male (kg)	40.65 (7.00)	36.12 (5.68)	$p = .079$	43.69 (5.66)	37.34 (5.27)	$p = .006$*
Hand-grip Strength Female (kg)	27.17 (4.71)	25.22 (3.28)	$p = .361$	26.17 (4.70)	26.08 (3.44)	$p = .768$
Social and Cognitive Activities:						
Social Network[b]	20.07 (4.74)	20.09 (6.24)	$p = .992$	20.95 (4.30)	19.00 (7.19)	$p = .728^{\Omega}$
Cognitive Activities[c]	46.48 (10.49)	50.89 (6.81)	$p = .114^{\ddagger}$	48.86 (8.24)	50.48 (7.60)	$p = .434^{\Omega}$

Note: a. Physical Activity Scale for the Elderly score. b. From Lubben Social Network Scale. c. From Florida Cognitive Activity Scale. * - $p < .05$. ‡ - From 1000-sample bootstrap results. Ω – From mixed ANOVA on trimmed means.

B.3.2 Experimental Tasks

Speed of Processing

Results are shown in Table B.3: Speed of Processing.

At the initial time point there were no significant differences between groups for the Trail Making Test part A (Trails A) or Symbol Copy task.

When including the follow-up time point in analyses, there were no main effects of group or time point nor a group by time point interaction for Trails A, *Qs* < 1, nor for Symbol Copy, *Fs* < 1 and $F(1, 38) = 2.35$, $p = .133$, respectively.

Executive Function

Results are shown in Table B.3: Executive Function.

For the DSST, indexing the time for higher mental function, there was no difference between groups. At the initial time point, for Trails B, accounting for motor speed (subtracting time taken for Trails A), there was no main effect between groups.

When including the follow-up time point in the analysis, there was again no main effect between groups, no main effect of time point, nor group by time point interaction; for the DSST, *Fs* < 1; for Trails B – A, *Qs* < 1 and $Q = 1.18$, $p = .291$, respectively.

Memory

Incidental and Episodic Memory:

Results are shown in Table B.3: Memory.

At the initial time point, there were no main effects between groups for incidental memory performance (from the DSST) nor for episodic memory performance.

When including the follow-up time point in the analyses, there was again no main effect between groups, no main effect of time point, and no group by time point interaction; for incidental memory performance, $F(1, 34) = 1.46$, $p = .236$, and $F(1, 34) = 1.46$, $p = .236$, respectively; for episodic memory, all *Fs* < 1.

Working Memory:

Results shown in Table B.3: Memory.

At follow-up, there was no main effect between groups for the Backwards Digit Span.

Prospective Memory:

Subjective Rating of Memory

Results for this measure are shown in Table B.3: Memory.

For the Prospective and Retrospective Memory Questionnaire, there were no differences between groups for ratings by supervets and controls of their own prospective and retrospective memory.

Including the follow-up in analyses, there was again no difference between groups, no difference between time points, and no group by time point interaction; for prospective memory, $Q < 1$ and $Q = 1.03$, $p = .319$, respectively; for retrospective memory, $Q = 1.26$, $p = .272$ and $Q < 1$, respectively.

Table B.3

Means, Standard Deviations, and Significances Between Supervet and Control Groups on Speed of Processing and Cognitive Tasks

Measure	Time Point 0 Supervets Mean (SD)	Time Point 0 Controls Mean (SD)	Significance at T0	Time Point 1 Supervets Mean (SD)	Time Point 1 Controls Mean (SD)	Significance with T0 and T1
Speed of Processing:						
Symbol Copy (secs/item)[a]	0.91 (0.19)	0.91 (0.17)	$p = .964$	0.89 (0.14)	0.85 (0.14)	$p = .909$
Trails A (secs)[b]	30.95 (6.88)	31.90 (11.33)	$p = .971$	31.74 (6.67)	32.17 (13.04)	$p = .963^{\Omega}$
Executive Function:						
DSST Higher Mental Function (secs/item)[c]	1.12 (0.28)	1.00 (0.31)	$p = .167$	1.09 (0.32)	1.03 (0.27)	$p = .385$
Trails B - A (secs)[d]	39.90 (27.03)	26.32 (12.55)	$p = .093$	35.63 (14.34)	23.14 (12.35)	$p = .500^{\Omega}$
Memory:						
Incidental Memory (percent recalled)[e]	41.20 (21.85)	48.15 (27.74)	$p = .382^{\ddagger}$	49.71 (25.76)	48.37 (23.55)	$p = .720$
Episodic Memory (percent recalled)	28.42 (13.44)	30.71 (11.91)	$p = .635^{\ddagger}$	28.33 (11.13)	26.15 (14.02)	$p = .859$
Backward Digit Spans (number of digits recalled)				5.76 (1.04)	5.95 (1.55)	$p = .673^{\ddagger}$
Subjective Prospective Memory[f]	19.01 (3.21)	19.00 (3.53)	$p = .991^{\ddagger}$	18.76 (2.86)	19.95 (3.11)	$p = .667^{\Omega}$
Subjective Retrospective Memory[f]	18.06 (3.95)	17.18 (3.17)	$p = .393^{\ddagger}$	18.14 (3.07)	18.59 (3.56)	$p = .603^{\Omega}$

Note: a. From Digit Symbol Substitution Task for 30 seconds. b. Trail Making Test part A time taken. c. Digit Symbol Substitution Task, time per item accounting for copying speed. d. Trail Making Test time taken for part B with part A subtracted. e. From Digit Symbol Substitution Task. f. From the Prospective and Retrospective Memory Questionnaire. ‡ - From 1000-sample bootstrap result. Ω – From mixed ANOVA on trimmed means.

Event-based PM Tasks

Card Sort Task:

Results for this task are shown in Table B.4.

Accuracy: PM

At the initial time point, there were no differences between groups (supervets / controls) in terms of PM performance.

Including the follow-up in the analysis, there was again no differences between groups, Q <1, no differences between time point, Q = 3.24, p = .085, and no significant group by time point interaction, Q <1.

Accuracy: Ongoing Card Sort Task

At the initial time point, accuracy for the No PM condition was not different between supervets and controls. When carrying a PM intention, however, supervets were less accurate than controls, $t(30.94) = 2.17$, $p = .038$.

Exploring further, supervets (M = 3.66%) had a higher accuracy cost of carrying a PM intention than controls (M = 1.09%), M_{diff} = 2.66, 95% CI [0.33, 4.86], p = .031.

When including the follow-up time point in the analyses, there was no difference between groups by time point, or group by time point interaction, either for the No PM condition, Qs < 1, or when carrying a PM intention, Q = 1.61, p = .217, and Q < 1, respectively.

Reaction Time: Ongoing Card Sort Task

At the initial time point, there were no RT differences between groups in either condition.

When including the follow-up time point in analyses, there were again no RT differences between groups; for the No PM condition, no main effect, no effect of time point, Q = 2.44, p = .132, and no group by time point interaction, Q < 1; for PM condition, Q = 1.65, p = .211, and Q < 1, respectively.

Table B.4

Means, Standard Deviations, and Significances Between Supervet and Control Groups on the Card Sort PM Task

	Time Point 0			Time Point 1		
Condition	**Supervets Mean (SD)**	**Controls Mean (SD)**	**Significance at T0**	**Supervets Mean (SD)**	**Controls Mean (SD)**	**Significance with T0 and T1**
PM Accuracy (%)	77.98 (28.75)	84.38 (18.53)	$p = .410^{‡}$	92.19 (12.81)	90.41 (11.29)	$p = .993$
Card Sort Accuracy without PM (%)	97.36 (2.27)	97.44 (2.38)	$p = .924^{‡}$	97.16 (2.89)	98.18 (1.68)	$p = .568^{Ω}$
Card Sort Accuracy with PM (%)	93.87 (4.34)	96.22 (2.33)	$p = .038^{*}$	95.51 (3.12)	95.75 (4.19)	$p = .156^{Ω}$
Card Sort Reaction Time without PM (ms)	557.55 (77.23)	548.99 (61.13)	$p = .722$	566.23 (77.46)	566.01 (62.64)	$p = .547^{Ω}$
Card Sort Reaction Time with PM (ms)	697.37 (78.85)	676.20 (77.62)	$p = .335$	713.42 (69.25)	702.67 (102.32)	$p = .847^{Ω}$

Note: * - $p < .05$. ‡- From 1000-sample bootstrap result. Ω – From mixed ANOVA on trimmed means.

McDaniel PM Task:

Results for this task are shown in Table B.5.

Accuracy: PM

We calculated the accuracy for each participant for both the focal and non-focal PM task. Late responses, almost always one trial after the PM cue, were coded as correct. This happened on 14 occasions at the initial time point, and 7 occasions at follow-up.

There was no significant difference between groups (supervets/controls), regardless of condition (focal PM/non-focal PM), $F(1, 38) < 1$.

Regardless of group, more PM targets were correctly identified in the focal PM condition than the non-focal PM condition, $F(1, 38) = 9.14$, $p = .004$.

There was no significant interaction between group and PM condition, $F < 1$.

When including the follow-up time point in the analysis, again there was no difference between groups, $F < 1$. There again was a main effect of PM condition, with more PM targets correctly identified in the focal PM condition ($M = 90.1\%$) than the non-focal PM condition (*M*

= 66.4%), $F(1, 28) = 17.52$, $p < .001$. There was again no interaction between group and PM condition, $F < 1$.

There was no main effect of time point, nor any interactions between time point and other factors, $Fs < 1.5$.

Accuracy: Ongoing Category Decision Task

For the ongoing task, the accuracy between groups (supervets/controls) was not significantly different, $F < 1$.

There was no significant difference in accuracy between PM conditions, $F < 1$.

There was also no significant interaction between group and PM condition, $F < 1$.

When including the follow-up in the analysis, there again were neither significant main effects nor interactions (all $Fs < 1.77$).

Reaction Time: Ongoing Category Decision Task

Reaction times for the ongoing task were not significantly different between groups, $F < 1$.

Reaction times between the PM conditions, as expected, were significantly different, $F(2, 76) = 70.65$, $p < .001$; sidak-corrected pairwise comparisons showed that reaction times for the no PM condition were faster than for the focal PM condition ($p = .004$) and non-focal PM condition ($p < .001$) and reaction times for the focal PM condition were faster than the non-focal PM condition ($p = .010$).

In addition, within-subjects contrasts found a condition by group linear interaction trend, $F(1, 38) = 3.61$, $p = .065$.

Sidak-corrected pairwise comparisons revealed controls' mean reaction times were comparable for the no PM and focal PM conditions ($p = .290$) while differences between all

other pairs were significant (*ps* < .001). Supervets, unlike controls, were significantly slower in the focal PM compared to the no PM condition (*p* = .011), as well as recording differences between all other pairs (*ps* < .001).

When including the follow-up in the analyses, there was again no difference between groups, *F* < 1, and there was again a significant difference between PM conditions, *F*(2, 56) = 106.20, *p* < .001. Trend analysis showed this to be a linear interaction, *F*(1, 28) = 133.65, *p* < .001, with reaction times increasing from the no PM (*M* = 1339.02) to the focal PM (*M* = 1457.02) and then to the non-focal PM (*M* = 1766.90) condition. There was a main effect of time point, *F*(1, 28) = 4.63, *p* = .040, with follow-up (*M* = 1493.91) being faster than the initial time point (*M* = 1548.05).

There was also a PM condition by group trend, *F*(1.60, 44.89) = 3.10, *p* = .066. The effect sizes for the PM condition comparisons were much larger for supervets than for controls. For the No PM vs. Focal PM comparison: *d* = 0.71 vs *d* = 0.34 for supervets and controls, respectively; for the No PM vs. Non-Focal PM comparison: *d* = 2.26 vs *d* = 1.00 for supervets and controls, respectively; for the Focal PM vs. Non-Focal PM condition: *d* = 1.72 vs *d* = 0.63 for supervets and controls, respectively.

There were no interactions with time point (all *Fs* < 1.33).

Table B.5

Means and Standard Deviations on the McDaniel PM Task for PM Accuracies, Ongoing Category Decision Task Accuracies, and Ongoing Category Decision Task Reaction Times

	Time Point 0		Time Point 1	
Task Condition	**Supervet Mean (SD)**	**Control Mean (SD)**	**Supervet Mean (SD)**	**Control Mean (SD)**
		PM Accuracy		
Focal PM (%)	89.39 (26.00)	88.89 (25.57)	91.67 (19.25)	97.62 (8.91)
Non-Focal PM (%)	62.12 (38.89)	70.37 (41.05)	56.25 (35.94)	76.19 (33.15)
		Ongoing Category Decision Task Accuracy		
No PM (%)	95.54 (2.31)	95.96 (2.42)	96.29 (2.54)	95.62 (2.73)
Focal PM (%)	95.83 (2.07)	95.75 (1.95)	95.92 (1.78)	93.64 (8.15)
Non-Focal PM (%)	95.62 (1.92)	95.12 (2.33)	95.17 (2.55)	95.48 (2.23)
		Ongoing Category Decision Task Reaction Time		
No PM (ms)	1396.81 (291.31)	1404.57 (316.10)	1239.52 (159.75)	1359.82 (323.64)
Focal PM (ms)	1519.76 (316.99)	1472.48 (319.86)	1395.84 (179.76)	1513.19 (422.99)
Non-Focal PM (ms)	1887.80 (431.72)	1732.57 (405.14)	1726.73 (269.58)	1728.35424.93)

B.4 Discussion

This study asked whether long-term high-effort endurance exercise may protect against the age-related declines reported in cognitive performance. We anticipated that supervets, defined as older adult volunteers with a 20+ year history of regular high-effort endurance exercise, would still have an advantage over our comparable group of volunteers not engaging in long-term high-effort endurance exercise, especially in measures that involve the selective deployment of limited capacity attentional resources in a prospective memory task.

Physiologically, while both groups had the same potential for fitness - as demonstrated by lung function measures, our supervet group was indeed more physically fit than our control group - as demonstrated by less percentage body fat and stronger hand-grip in males.

Cognitively, our analyses found surprisingly few differences between our two groups of supervets and non-sedentary controls at the initial time point and at both time points.[2] We found no differences between supervets and controls in speed of processing (Trails A and Symbol Copy), executive function (Trails B and DSST), working memory, and incidental memory, and episodic memory. On measures of prospective memory, differences did emerge.

With PM we anticipated that supervets would show smaller costs in carrying a PM intention, but that this was likely to manifest on non-focal PM tasks, based on the evidence that exercise increases attentional resources used for monitoring (Barnes et al., 2003; Voss et al., 2010; Angevaren et al., 2008, Smith et al. 2010; Erikson et al., 2011; Winker et al., 2010; Voss et al., 2010a). In the Card Sort Task, involving a non-focal PM cue, in fact supervets showed, instead, greater cost in carrying out the PM condition, indexed by poorer ongoing task accuracy. This difference was not sustained at the 12-month follow-up, however. This may relate to the novelty value of the task in the first session; we return to this point later in the discussion.

In the McDaniel Focal and Non-Focal PM Task, we also identified differences in performance by the two groups. Specifically, supervets, but not controls, showed a cost in carrying an intention in the focal PM condition of the task. This difference is particularly interesting. Einstein & McDaniel's (2000) multiprocess theory suggests that the less complex focal PM condition should rely more on spontaneous retrieval than monitoring, and therefore should show no cost in reaction time. Results from our controls are consistent with this suggestion.

One interpretation of the significant cost in reaction time in the focal PM condition for supervets could be that this group engages in active monitoring when there is any PM component, regardless of focality. The fact that this did not improve PM performance is consistent with findings of Einstein et al. (2005) in their high-emphasis instruction task.

[2] Multiple regression analyses using the cohort as a single continuous group and physical activity / physiological measures as predictors did not alter these outcomes.

Einstein et al. (2005) suggested that easier event-based PM tasks will only need spontaneous processing, but for PM tasks that are harder, have high-emphasis instructions, or have more PM targets: monitoring, which absorbs attentional resources, may be required. In one experiment, they found that in tasks with high-emphasis instruction, non-focal targets, or both of these, monitoring increased as evidenced by higher reaction time costs in the ongoing task. In terms of PM performance, increased monitoring due to high-emphasis instruction when paired with a focal PM task had minimal and non-significant effects. They attributed this to the use of spontaneous processes being enough for focal PM tasks and the additional monitoring being redundant. In contrast, the additional monitoring in a non-focal PM task resulted in a benefit to PM performance, which they explained in terms of spontaneous processes alone being insufficient for non-focal PM tasks. Importantly for their model, the focal target and moderate-emphasis instruction condition showed high PM performance without any significant cost to the ongoing task.

Marsh, Hicks, & Cook, (2005) further explored effort in the ongoing task in relation to focal and non-focal PM cues. For younger adults, they found that only when there was a focal PM cue was there decreased cue detection when higher effort was required for the ongoing task. When the PM cue was non-focal, ongoing task effort did not influence cue detection, despite slower reaction times overall in the ongoing task. So contrary to McDaniel and Einstein's (2000) multiprocess framework, they suggest focal PM cue detection is not always automatic, but that when there is high effort on the ongoing task, both the ongoing task and PM task compete for the same "pool" of resources. The present finding for the supervets, then, is consistent with Marsh et al.'s (2005) finding under high effort, and suggests that the supervets did not process the focal PM cue "automatically".

In both PM tasks completed here a parsimonious explanation of our results is that the supervets were self-imposing an increased importance on their performance, applying higher

effort in the form of monitoring, regardless of whether or not the added effort actually resulted in a performance benefit.

Previous literature has suggested that individual differences in personality style can influence performance on PM tasks (Cuttler et al., 2007; Salthouse et al., 2004). It is possible that this higher effort put in by the supervets reflects different personality styles between the groups, and this is something that we are currently exploring. Anecdotally, it was clear that the supervets were a highly motivated group who were a) much more keen to attend the 4 hour testing sessions and b) individually committed to the view that their exercise regimen improved their cognitive health and wellbeing. It is therefore doubly surprising that, with such motivation, they did not demonstrate superior performance across the task battery compared to the control group.

This explanation also fits with their performance on the card sort task, motivational differences for the first visit may have resulted in differential performance, but at follow-up familiarity and practice effects may have attenuated this initial difference.

Other recent studies of senior athletes (Tseng, Uh, et al., 2013; Winker et al., 2010); have reported significant benefits of exercise on cognition, but only in a relatively narrow range of executive function tasks amongst the many measures used.

One key difference between the present study and previous work was our selection of controls. Unlike the earlier studies, our controls were similar to supervets in social network (Richards, Hardy, & Wadsworth, 2003), cognitive activity (Sturman et al., 2005), and depression levels (Thomas & O'Brien, 2008), as well as being physically mobile and engaging in similar amounts of moderate physical activity as our supervets (Scherder et al., 2013). These may have reduced the differential effects previously seen.

A second difference might be the time frames of the studies. It is possible that the cognitive benefits of exercise may relate to relatively short-term interventions in previously sedentary groups, and that benefits are not sustained in extreme long-term exercisers. One possibility is that detrimental effects of intense activity, such as physiologically damaging oxidative stress (Radak, Chung, Koltai, Taylor, & Goto, 2008), may be offsetting any benefit exercise confers. This could explain why Tierney, Moineddin, Morra, Manson, & Blake (2010) in a cohort of post-menopausal women (aged 50-65) reported a positive relationship between moderate physical activity and one memory and two executive function tasks but also a negative relationship between strenuous physical activities and three memory and two executive functions tasks.

It should be noted, however, that careful review of the earlier literature actually indicates that the reported effects of exercise often fail to reach conventional levels of significance in individual papers. Meta-analyses are common and even they do not show very large effects. Some also contain confounds such as multiple representation of data from the same study and including genders from the same study separately. A thorough recent review that takes account of these methodological irregularities (Young et al., in revision) in fact reported that there is very little evidence for positive effects of exercise on cognition in older healthy adults.

In conclusion, and in contrast to earlier studies looking at short-term exercise interventions and at habitual moderate exercise, our comprehensive evaluation of long term high-effort endurance exercise suggests a different relationship with cognition. Logically, this makes sense because the beneficial effects of exercise cannot be continuous; there must be a ceiling beyond which additional exercise does not confer additional cognitive benefit. In fact the results of this study show clearly that supervets participating in long-term high-effort endurance exercise display only small cognitive differences relative to non-sedentary volunteers with a similar age, social, cognitive and neuropsychiatric profile. These small

differences manifest in tasks requiring application of effort and strategy, and potentially index differences in effort or motivation in the supervets.

In future studies, the effect of effort must be disentangled from the performance measures. This could be achieved by introducing an effort manipulation or an explicit index of individual effort differences in response to the cognitive task, to ensure that we can cleanly derive a measure of the effect of long-term high-effort endurance exercise on cognitive performance uncontaminated by potential personality differences.

Overall, however, the current work suggests that the benefits of long-term high-effort endurance exercise in the over 60s may be limited to increased physical fitness and strength, and may not extend to substantive improvements in cognitive performance, when comparison is relative to moderately active volunteers of similar age, social, cognitive and neuropsychiatric profile.

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C. **Article II:** Cognitive Effects of Long-Term High-effort Endurance Exercise in Older Adults: Are There Measurable Benefits to Attention?

Abstract

Aging is associated with reduced attentional resources and decline in attentional control. In attention tasks, exercise has been shown to benefit cognition especially in conditions that require more attentional control. However, the relationship between long-term high-effort endurance exercise and attention is unclear. Using a cross-sectional design at two time points, 12 months apart, we recruited older adults engaging in high-effort endurance exercise for at least twenty years, and compared them with a non-sedentary control group similar in age, sex, education, IQ, depression levels, and other lifestyle factors. We hypothesized that long-term high-effort endurance exercise would protect against age-related decline in attention giving better performance especially in harder conditions. Our findings of subtle differences in the most complex attention tasks indicated possible strategic and motivational differences between groups. However, overall and in contrast to previous studies, there was very little cognitive difference between those participating in long-term high-effort endurance exercise and non-sedentary control volunteers.

C.1 Introduction

Older adults have more difficulty than younger adults with tasks that are more complex, effortful, and strategic (Luo & Craik, 2008; Salthouse, 2010). Luo & Craik (2008) summarize the models for these age-related changes as: a slowing of processing speed, which plays a role in other cognitive functions; reduced attentional processing resources; loss of inhibitory functions; and decline in controlled processing, such as recollection, in contrast to spared automatic processing, such as familiarity.

Focusing on attention, its study has been divided into three main aspects: selectivity, intensity, and executive attention (McAvinue et al., 2012; Parasuraman, 1998; A. Raz & Buhle, 2006). In our study, our main attention tasks of visual search, sustained attention, and attentional control respectively belong to these aspects. Aging affects these three aspects of attention differentially; we detail the effects in the following, focusing specifically on the types of tasks utilized in our study.

C.1.1 Visual Search

Much research has explored aging and visual search in the past, offering a number of explanations for the age-related decline observed. For example, Rabbitt (1965) using a visual search task with varying number of irrelevant stimuli, observed that older adults took more time to ignore irrelevant stimuli than younger adults resulting in older adults' worse task performance. Later Plude & Doussard-Roosevelt (1989), comparing younger and older adults with a task able to differentiate between feature extraction and feature integration (also called feature conjunction or binding), observed that older adults overall had slower reaction times and increased errors as number of items displayed increased, especially in feature integration trials. Interestingly the number of items in the feature extraction only condition did not affect reaction times for both age groups. The authors interpreted these results as age-related

decline being explained then by decrements to feature integration and not feature extraction with the overall slower reaction time being explained by general slowing (Salthouse, 1996, 2000). Trick & Enns (1998) looking cross-sectionally at visual search task performance on a number of visual search tasks, observed little age-related differences, including integration of two features, but did observe that older adults were less able to move attention voluntarily from item to item than younger adults. Therefore there may be a number of mechanisms that account for age-related decline in visual search including increased time in ignoring irrelevant stimuli, decrement in feature integration ability, general slowing, and decrement in item to item attentional movement.

C.1.2 Sustained Attention

Turning to sustained attention tasks, one widely used task is the Rapid Visual Information Processing (RVIP; Wesnes & Warburton, 1983) task which also involves working memory and fatigue because of its length. In this task participants see a sequence of single digit numbers appearing one at a time in the middle of the screen. When sequences of three even numbers or three odd numbers appear in a row, the participant makes a button press. In this task it has been observed that performance in reaction time and accuracy declines with age (Pagnoni & Cekic, 2007). Also this task was sensitive enough to detect differences from meditation (Pagnoni & Cekic, 2007), caffeine (Smit & Rogers, 2000), and nicotine (Lawrence, Ross, & Stein, 2002), which all have been observed to have a beneficial effect on RVIP performance. However in another sustained attention task, a high-event rate digit discrimination task with varying levels of stimulus degradation, where participants were screened for optimal physiological health, the authors observed no effect of age on accuracy or reaction time. The authors explained their result may have been due to only including those with optimal health in their study who were less likely to have deficits with increasing age (Berardi, Parasuraman, & Haxby, 2001). Other cross-sectional studies of older and younger adults though have observed

differences in sustained attention tasks. Parasuraman, Nestor, & Greenwood (1989) and Deaton & Parasuraman (1993) observed in similar digit discrimination tasks with stimulus degradation that as processing demands increased due to increased degradation, differences between age groups in overall sustained attention levels became larger, with older adults having lower levels. Interestingly in the Parasuraman et al. (1989) study, older adults, with their presumed more limited processing resources, were able to compensate at the lowest processing demand condition to display similar performance in hit rate as younger adults; however with increased processing demand, older adults were no longer able to compensate to the same levels, performing worse due to having less processing resources.

In an effort to account for processing demands, Tomporowski & Tinsley (1996) equated for working memory load in a sustained attention task while exploring intrinsic and extrinsic motivation. The authors observed that when not expecting payment (extrinsic motivation), older adults performed better than younger adults, in contrast to previous results. When two separate groups of younger and older adults instead expected payment, the performances of both groups were similar. The authors concluded that when equating for working memory load, normal aging effects are no longer observed. The authors also suggested that older adults had more intrinsic motivation for their performance than younger adults, and this additional intrinsic motivation resulted in the older adults performing better than younger adults when not expecting payment.

Motivation in attention tasks has been touched upon previously. Yeh & Wickens (1988) theorized that motivational factors were able to produce dissociations between performance and perceived workload. Specifically in sustained attention tasks, Bunce & Sisa (2002) observed that middle-aged to older adults perceived a greater increase in workload than younger adults, but this did not affect their performance. Earlier, Deaton et al. (1993) when

manipulating workload and type of sustained attention task observed that both young and old participants displayed better performance in a higher perceived workload but interesting task than a lower perceived workload but less interesting task. Their results lend support to Yeh & Wickens's (1988) theory: interest motivated participants to expend more attentional resources and thereby improve their performance. Therefore, it seems that when participants are not as interested in a task, they do not fully utilize their attentional resources for task performance.

So at least for sustained attention tasks, in addition to processing resources, motivation and interest seem to play a role in performance.

C.1.3 Attentional Control

Attentional control or executive attention is used in goal-driven behavior where one responds to certain stimuli and may need to ignore others (Astle & Scerif, 2009; Posner & Petersen, 1990). Performance on many types of cognitive tasks then is mediated by attentional control, for example task-switching and interference tasks. In the literature however, separation of attentional control from executive function is unclear as their definitions overlap and the same tasks have been associated with both (Posner & Petersen, 1990; Posner & Snyder, 1975). Here we chose to call these attentional control tasks.

With aging, Coubard et al. (2011) in a cross-sectional study observed that attentional control declined with age: older adults were worse than young adults at suppressing attention, switching attention for unpredictable events, and preparing for unpredictable events. Interestingly, older adults were not worse than young adults at switching attention for predictable events though. The authors also found that between-participant variability increased with age, meaning perhaps that non-age-related factors could also be at play during attentional control task performance. In terms of within-participant variability, older adults

had more reaction time variability than younger adults. This within-participant RT variability can be used as an index of the stability of attentional control (West, Murphy, Armilio, Craik, & Stuss, 2002). Past research including simple and choice reaction time tasks have also observed RT variability to increase with age (Anstey, 1999; Bunce, MacDonald, & Hultsch, 2004; Hultsch, MacDonald, & Dixon, 2002; MacDonald, Hultsch, & Bunce, 2006; West et al., 2002). Bunce et al. (2007) observed that more frontal lobe white matter hyperintensities (WMH) were associated with increased RT variability, suggesting that deterioration of neural pathways in the frontal lobe could be responsible for the age-related increase in RT variability, supporting previously proposed mechanistic theories (MacDonald et al., 2006).

Another attentional control measure we were interested in was the Stroop task, assessing cognitive inhibition. There is some debate regarding age-related performance decline in Stroop performance. Some evidence from Verhaeghen & De Meersman's (1998) meta-analysis found no difference between age groups in Stroop interference effect, calculated as the mean standard difference between neutral and interference conditions. The authors suggested then that declines seen in Stroop interference are just attributable to age-related declines in processing speed, where the interference condition naturally requires more processing time than neutral conditions. This evidence lent support to Salthouse (1996), who previously observed that all Stroop conditions loaded highly on one speed factor. More recent studies though suggest that declines in cognitive inhibition are distinct from other age-related declines (Bugg et al., 2007, as cited in Wolf et al., 2013) and have a neurobiological basis: Stroop interference performance has been shown to be associated with white matter integrity in frontal pathways (Wolf et al., 2013).

A newer attentional control measure has combined the traditional Stroop task with a task-switching paradigm: the Stroop-switch task (Hutchison et al., 2010). Here trials are intermixed

requiring either color-naming or word-naming, with the naming condition switching every two trials. The stimuli comprised two interference types: 1) incongruent “color words”, where the ink color of the word did not match the color the word spelled, eliciting the traditional Stroop interference effect of slower reaction times and higher error rates; 2) “neutral words”, non-color words that are matched to the “color words” in phoneme characteristics and printed word frequency. This measure was used to strongly discriminate between healthy older adults and those with very mild DAT, where those with very mild DAT had greater incongruent color word error rates. This discrimination was better than 18 standard psychometric tests. The task also discriminated between younger and older adults in incongruent color word error rates as well.

C.1.4 Exercise

One factor that may account for some of the increased variability in cognitive task performance in older adults, alluded to by Coubard et al. (2011), is exercise. Exercise in older adults is associated with less cognitive impairment and better cognitive performance in domains including global processing, working memory, attention, and executive function in retrospective and longitudinal studies (Barnes et al., 2003; Middleton et al., 2010; Weuve et al., 2004). Furthermore, systematic reviews and meta-analyses of Randomized Control Trials (RCTs) lasting 2 months to 6 years, showed that exercise improved cognitive performance in sedentary adults, including motor function, processing speed, memory, executive function, and attention (Angevaren et al., 2008; Colcombe & Kramer, 2003; Etnier et al., 1997; Hindin & Zelinski, 2012; P. J. Smith et al., 2010).

C.1.5 Exercise and Attention

Much of the exercise literature, summarized in the above, has shown benefit of exercise including in attention and executive function tasks, the latter also corresponding to attentional control tasks as previously noted.

Looking at the effects on individual attention tasks, for example using a sustained attention task, Bunce, Barrowclough, & Morris (1996) in middle-aged to older adults and younger adults, observed that physical fitness attenuated the age-related decline in performance on the task. In a follow-up Bunce (2001) used younger and older participants and this time manipulated task demands by degrading stimuli. Again fitness attenuated age-related decline, especially when high demand was placed on attentional resources.

In Stroop tasks, Prakash et al. (2011) using a modified Stroop task, observed that higher levels of cardiorespiratory fitness were associated with better performance in their most challenging condition. The authors suggested that increased cardiorespiratory fitness enhanced function by enhancing attentional control.

In the two meta-analytic reviews of exercise interventions that separated out Stroop interference (Angevaren et al., 2008; P. J. Smith et al., 2010), no differences from exercise interventions were found. Recently though, Predovan, Fraser, Renaud, & Bherer (2012) in a 3-month aerobic exercise RCT with sedentary older adults, used a paper-and-pencil Stroop task that included a harder condition block: in addition to naming the color of incongruent stimuli, on 20% of the trials the task switched to reading the word instead. The authors observed that only on this harder condition did their aerobic training group significantly improve their performance more than their controls in both reaction time and error rates. Also the amount of increase in aerobic capacity for the training group was negatively correlated with post-intervention reaction time in the harder condition. Therefore, a benefit of exercise was

detected in a more difficult random switch condition of the Stroop task, demonstrating potentially an exercise-related increase in attentional control in older adults.

More recently, research with older endurance athletes to explore the long-term effect of exercise have been undertaken. Winker et al. (2010) studied elderly marathon runners and bicyclists against sedentary older adults similar in age, sex, and education. They observed that their athletes trended better in the Stroop task. Tseng, Uh, et al. (2013) comparing a small group of twelve older runners with twelve sedentary volunteers, controlling for IQ differences, found their runners did not perform better than sedentary controls in the Stroop task.

In summary, aging is related to reduced attentional processing resources, resulting in age-related deficits in visual search, sustained attention; as well as decline in attentional control. Exercise seems to selectively benefit task conditions that require more processing resources or attentional control. For studies with long-term exercisers the relationship with attention is unclear, therefore further exploration is necessary.

The current study engaged two older adult populations that were distinctly different in their exercise profiles. The first group, super veteran athletes (“supervets”) as categorized by UK Athletics, was engaging in long-term (minimum 20 years) high-effort endurance exercise. The second group, non-sedentary control volunteers, was similar in age, gender, and full-time education to the supervet group, while socially active but not exercising beyond regular levels. With these populations we set out to test the effects of long-term high-effort endurance exercise in older adults on attentional task performance longitudinally.

C.2 Methods

C.2.1 Ethics Statement

Ethical approval was obtained from the University of Sussex Life Sciences & Psychology Cluster based Research Ethics Committee. Written consent was obtained from all participants.

C.2.2 Participants

For inclusion in the study, we chose non-smoker (never smoked or not smoked in the past 5 years) participants aged 60-85, on stable medication if any during the past 12 months, with English proficiency equivalent to that of a native speaker.

For our supervet group we required active exercise at a high level mostly via running, swimming, and/or cycling (self-paced sports) for the past 20 or more years. We chose only self-paced sports because athletes of interceptive-dominant sports, which usually involves a part of the body or a stick or racquet to interact with a ball, have been shown to have faster reaction times (Voss et al., 2010b), which alone could have an effect on cognitive performance. Also we chose high-effort endurance exercise to differentiate from exercise consisting of just short high-effort bursts or sustained low effort.

We excluded participants if they had a history of stroke, myocardial infarction, recently diagnosed diabetes, very high blood pressure (systolic above 200 and diastolic above 100), psychiatric or neurological disorders (self-reported), or were suffering from clinical depression.

We used the Mini Mental State Exam (MMSE; Folstein, Folstein, & McHugh, 1975), which assesses general cognitive function to exclude for Mild Cognitive Impairment and Dementia. In addition we used the Geriatric Depression Scale (GDS; Sheikh & Yesavage, 1986), which rates symptoms of depression, to exclude for depressed individuals.

We recruited 27 supervets mainly from running and triathlon clubs, but also from park runs, the finish line of races, running shops, as well as word of mouth. We recruited 23 control volunteers from churches, old age associations, women's clubs, university alumni associations, and word of mouth. This was designed to ensure a match for the social interaction that many of the supervets obtained from their participation in the running/triathlon clubs.

We excluded from analysis those diagnosed with a memory disorder at follow-up, resulting in one control volunteer being excluded, leaving 22 control volunteers.

Participants were tested at the University of Sussex campus and were compensated for their transportation costs and parking.

C.2.3 Demographics

Descriptions of demographic measures have been detailed in a previous paper (Article I). In brief we took measures of physical activity, diet, lung function, percent body fat, hand-grip strength, full-scale IQ, frequency of cognitive activities, and social network.

C.2.4 Experimental Tasks

Speed of Processing

We used the Simple Reaction Time (SRT) task (Bunce, Handley, & Gaines, 2008), here participants made a button press as soon as they could while maintaining accuracy when they saw a fixation cross turn into an "X" which was presented at a randomly determined interval between 300 and 1000ms. This task consisted of 8 practice trials and 48 test trials. The computer recorded the reaction time to each button press. The SRT can be used for calculating

intra-individual RT variability. We calculated measures of Intra-Individual Variability (IIV) and Intra-Individual Coefficient of Variability (ICV), which takes into account the mean reaction time of participants.

Attention

1. The Map Test of Everyday Attention (Map TEA; Robertson, Ward, Ridgeway, & Nimmo-Smith, 1996) is a time-limited visual search task, which has a visual selective attention component and contains a speed of processing element. In this task participants circle a specific symbol on a map for 2 minutes. We used a modified version with our participants, which contained multiple types of symbols on the map to make the task harder.

2. Rapid Visual Information Processing (RVIP; Wesnes & Warburton, 1983)

The computer recorded responses and reaction times. In our version we presented one stimulus per second. We had five 80-second intervals, with eight target sequences presented in each interval. The total time for the task was 6:20.

3. The Stroop-Switch Task (Hutchison et al., 2010)

Here trials are intermixed requiring either color-naming or word-naming prompted by a differing cue, with the naming condition switching every two trials. Therefore there were two response conditions: 1) color - to name the ink color, ignoring the word itself, 2) word - conversely to name the word, ignoring the color of the word. Also the task-switching component therefore also had 2 conditions: 1) switch trials – the task for the current trial is different from the previous trial; 2) non-switch trials – the task for the current trial is the same as the previous trial. The stimuli comprised two interference types: 1) incongruent “color words”, where the ink color of the word did not match the color the word spelled, eliciting the traditional Stroop interference effect of slower reaction times and higher error rates; 2)

"neutral words", non-color words that are matched to the "color words" in phoneme characteristics and printed word frequency. This task was self-paced with trials advancing after detecting the participants' voice. There were 24 practice trials and 88 test trials. The computer recorded reaction times, while the experimenter recorded errors.

C.2.5 Procedure

Participants completed the 3-hour session of cognitive and physiological testing in the following order: 10 minutes greet and consent, 15 minutes physiological measures, 75 minutes cognitive measures, 20 minutes tea break, 30 minutes cognitive measures, and 30 minutes questionnaires. Only a subset of the cognitive measures are reported in this paper.

C.2.6 Preliminary review of data, and data exclusions

Stroop-Switch Task

At the initial time point, one supervet was excluded because the participant did not complete the task properly. At follow-up one supervet was excluded because the participant did not complete the task properly.

RVIP

At the initial time point, one control was removed because the participant did not complete the task properly; one set of data was lost due to computer error. At follow-up there were no exclusions.

C.2.7 Statistical Analysis

Demographics & Experimental Tasks

For demographics, we used independent t-tests to compare means of measures between groups (supervets vs. controls) at each time point. For other measures at the initial time point we used independent t-tests between groups to compare means of measures where appropriate. ANOVAs were used if a task had a repeated measures factor. Follow-up analyses used mixed factor ANOVAs with time point as a repeated measures factor. For all statistical tests an alpha of .05 was adopted.

C.3 Results

C.3.1 Demographics

Demographic data was presented previously (see Article I). In brief, there were no significant differences between groups in sex, age, education, IQ, or depression levels. Supervets took part in more physical activities than controls, especially strenuous sports and muscle strength / endurance activities. There were no differences between groups in adherence to Mediterranean diet (only initial time point analyzed). Supervets and controls had the same potential for fitness as displayed by no differences in lung function measures. There were no differences between groups in social and cognitive activities.

Physiologically supervets had less percent body fat than controls and male supervets had stronger hand-grip than male controls.

C.3.2 Experimental Tasks

Speed of Processing & Variability

Results are shown in Table C.1: Speed of Processing.

At the initial time point there were no significant differences between groups for the mean reaction time for correct trials in the SRT task, IIV, or ICV between groups.

When including the follow-up time point in analyses, for the SRT there was no main effect of group, $F(1, 38) = 3.59$, $p = .066$, time point, $F(1, 38) = 1.48$, $p = .232$, nor a group by time point interaction, $F < 1$. For IIV, again there was no main effect of group for IIV. IIV was significantly higher at follow-up than at the initial time point, $F(1, 38) = 4.21$, $p = .047$, and there was no group by time point interaction, $F < 1$. For ICV there was no main effect of group, time point, $F(1, 38) = 3.14$, p = .085, nor a group by time point interaction, $F < 1$.

Attention tasks

Map TEA:

Results are shown in Table 1: Attention.

There was no significant difference between groups on the Map Test of Everyday Attention.

When including the follow-up time point in analyses, there was again no main effect of group, time point, $Q = 3.91$, $p = .061$, nor a group by time point interaction, $Q < 1$.

Table C.1

Means, Standard Deviations, and Significances between Supervet and Control Groups on Speed of Processing and Cognitive Tasks

	Time Point 0			Time Point 1		
Measure	**Supervets Mean (SD)**	**Controls Mean (SD)**	**Significance at T0**	**Supervets Mean (SD)**	**Controls Mean (SD)**	**Significance with T0 and T1**
Speed of Processing:						
Simple Reaction Time (ms)	301.71 (48.65)	326.29 (52.62)	$p = .093$	300.78 (48.80)	333.95 (92.05)	$p = .066$
IIV (SD, ms)[a]	78.64 (26.10)	81.75 (29.03)	$p = .695$	94.09 (39.30)	86.59 (41.92)	$p = .734$
ICV (SD/mean * 100)[b]	25.63 (7.21)	24.55 (6.98)	$p = .599$	30.39 (10.92)	25.23 (9.06)	$p = .090$
Attention:						
Visual Search (total no. correct)[c]	57.89 (12.93)	56.09 (9.32)	$p = .587^{‡}$	56.90 (12.28)	54.05 (10.84)	$p = .936^{Ω}$

Note: a. Intra-individual Variability. b. Intra-individual Coefficient of Variability. c. From Map Test of Everyday Attention. ‡ - From 1000-sample bootstrap result. Ω – From mixed ANOVA on trimmed means.

RVIP:

Results for this task are shown in Table C.2.

Number of Correct Responses

There was no significant difference between groups for number of correct responses $F(1, 46) < 1$. There was a significant difference across bins $F(4, 184) = 8.11$, $p < .001$. Trend analysis showed this to be a linear relationship $F(1, 46) = 18.73$, $p < .001$. There was no significant bin by group interaction, $F(4, 184) < 1$.

When including the follow-up time point in the analysis, there was again no main effect of groups, again a significant difference across bins, $F(4, 140) = 12.37$, $p < .001$, this time qualified by a bin by group interaction, $F(4, 140) = 2.78$, $p = .029$. Sidak-corrected pairwise comparisons showed that supervets correctly identified significantly more sequences in the first bin than

any of the other bins, (*ps* = .001 or less), while the rest of the bins did not differ significantly in the number correct. For controls, they correctly identified significantly more sequences in both the second fourth bin than in the fifth bin, $p = .031$ and $p = .009$ respectively.

Reaction Time

For the average reaction times of correct responses, at the initial time point there was no main effect of group, $F < 1$, bin, $F(4, 180) = 2.37$, $p = .055$, but a significant group by bin interaction, $F(4, 180) = 2.55$, $p = .041$. Sidak-corrected pairwise comparisons showed that reaction time differences across bins were limited to supervets, where the first bin was significantly faster than the second bin ($p = .004$), while the rest of the bins were not significantly different from each other.

Including the follow-up time point, there was again no main effect of group, $F < 1$, nor time point, $F(1, 34) = 2.22$, $p = .146$. There was a significant difference between the bins $F(4, 136) = 7.90$, $p < .001$, qualified by a group by bin interaction, $F(4, 136) = 2.87$, $p = .025$. Sidak-corrected pairwise comparisons showed that reaction time differences across bins were between groups: for supervets the first bin was faster than the second ($p < .001$), fourth ($p = .009$), and fifth bin ($p = .005$); for controls the first 3 bins were faster than the fifth bin ($p = .019$, $p = .042$, $p = .030$ respectively).

Table C.2

Means and Standard Deviations for RVIP Task, Number of Correct Responses and Reaction Times (ms)

	Time Point 0				Time Point 1			
	Supervets		Controls		Supervets		Controls	
Bin	Correct (SD)	RT (SD)	Correct (SD)	RT (SD)	Correct (SD)	RT (SD)	Correct (SD)	RT (SD)
1	6.73 (1.37)	492.42 (63.46)	6.59 (1.79)	519.36 (80.17)	7.19 (0.75)	491.61 (61.21)	6.31 (1.89)	511.30 (72.91)
2	5.50 (2.18)	530.88 (64.54)	6.36 (2.13)	508.41 (75.77)	5.81 (1.40)	530.41 (66.31)	6.31 (2.21)	529.26 (56.10)
3	5.23 (1.86)	523.36 (77.11)	5.59 (1.92)	511.91 (72.07)	6.00 (1.58)	522.30 (75.73)	6.19 (2.34)	518.85 (64.30)
4	5.73 (1.37)	524.55 (52.13)	5.82 (1.76)	530.25 (75.12)	5.95 (1.36)	533.53 (76.36)	6.19 (2.26)	529.65 (75.14)
5	5.31 (2.09)	523.00 (52.81)	5.55 (2.26)	537.39 (87.84)	5.48 (1.57)	529.61 (72.74)	5.06 (2.29)	569.38 (90.75)

Stroop Switch Task:

Results for this task are shown in Table C.3.

Errors

At the initial time point, switch trials produced more errors than non-switch trials, $F(1, 46) = 5.38$, $p = .025$. Color naming produced significantly more errors than word naming, $F(1, 46) = 19.22$, $p < .001$, and incongruent trials produced more errors than neutral trials, $F(1, 46) = 7.75$, $p = .008$, but these were both qualified by a significant interaction of cue and interference, $F(1, 46) = 41.42$, $p < .001$. Sidak-corrected pairwise comparisons confirmed that error differences were limited to incongruent trials only, with more errors occurring during color-naming trials ($p < .001$).

Including the follow-up included in the analysis, color naming again produced more errors than word naming, $F(1, 36) = 18.39$, $p < .001$, qualified by a cue by switch interaction, $F(1, 36) = 5.01$, $p = .031$: only in word-naming trials did switch trials have higher error rates than non-switch trials, $F(1, 36) = 8.54$, $p = .006$.

Again, incongruent trials produced more errors than neutral trials, $F(1, 36) = 15.73$, $p < .001$, again qualified by a cue by interference interaction, $F(1, 36) = 48.02$, $p < .001$: error differences were limited to incongruent trials, where color naming produced more errors than word naming, $F(1, 36) = 39.93$, $p < .001$.

There was a group by time point interaction, $F(1, 36) = 4.37$, $p = .044$, qualified by a group by time point by cue interaction, $F(1, 36) = 8.04$, $p = .007$: sidak-corrected pairwise comparisons confirmed that only in supervets at the follow-up time point did color naming produce more errors than word naming ($p = .008$).

Reaction Time

Color naming was slower than word naming, $F(1, 46) = 139.32$, $p < .001$, and incongruent trials were slower than neutral trials, $F(1, 46) = 28.72$, $p < .001$. Both were qualified by a significant interaction of response cue and interference, $F(1, 46) = 11.63$, $p = .001$: sidak-corrected pairwise comparisons confirmed that reaction time differences were limited only to color-naming trials, with slower incongruent than neutral trials ($p < .001$).

There was a cue by switch interaction, $F(1, 46) = 4.99$, $p = .030$, qualified by a group by cue by switch interaction, $F(1, 46) = 5.92$, $p = .019$: sidak-corrected pairwise comparisons confirmed that reaction time difference were limited to word-naming trials, where 1) supervets were faster than controls in switch trials only ($p = .046$) and 2) switch trials were slower than non-switch trials for controls only, $F(1, 21) = 30.89$, $p < .001$.

When including follow-up in analyses, color naming was slower than word naming, $F(1, 36) = 167.83$, $p < .001$, and again incongruent trials were slower than neutral trials, $F(1, 36) = 34.39$,

p < .001, these were again qualified by a cue by interference interaction, $F(1, 36) = 20.43$, p < .001.

The initial time point was slower than the follow-up time point, $F(1, 36) = 7.73$, $p = .009$, and switch trials were slower than non-switch trials, $F(1, 36) = 4.81$, $p = .035$. There was a cue by switch interaction, $F(1, 36) = 9.60$, $p = .004$, qualified by a group by cue by switch interaction, $F(1, 36) = 4.65$, $p = .038$, qualified by group by cue by switch by time point interaction, $F(1, 36) = 8.03$, $p = .007$, attributable to the initial time point already described.

Table C.3

Means and Standard Deviations for Stroop-Switch Task Reaction Times (ms) and Percent Errors

	Time Point 0				Time Point 1			
	Supervets		Controls		Supervets		Controls	
Condition	Mean RT (SD)	Mean % Error (SD)	Mean RT (SD)	Mean % Error (SD)	Mean RT (SD)	Mean % Error (SD)	Mean RT (SD)	Mean % Error (SD)
Color:								
Incongruent:								
Switch	1020.13 (303.15)	14.38 (16.06)	1018.68 (268.01)	18.63 (18.87)	925.13 (181.06)	12.56 (17.08)	986.26 (169.35)	11.17 (11.34)
Non-Switch	1035.75 (306.19)	14.96 (15.81)	1037.82 (219.88)	16.47 (15.07)	952.75 (186.47)	19.93 (18.61)	997.00 (202.16)	10.14 (8.02)
Neutral:								
Switch	912.16 (255.06)	6.65 (10.49)	926.89 (191.37)	9.52 (9.42)	811.12 (153.16)	8.26 (17.80)	876.61 (129.08)	1.93 (4.45)
Non-Switch	870.95 (210.24)	4.83 (10.18)	940.57 (219.85)	4.77 (9.02)	852.27 (168.91)	8.00 (13.75)	881.00 (193.76)	2.36 (4.58)
Word:								
Incongruent:								
Switch	795.40 (182.97)	3.55 (7.16)	878.62 (211.71)	2.34 (5.46)	755.82 (191.29)	3.75 (6.33)	829.84 (209.95)	5.03 (5.60)
Non-Switch	778.62 (240.93)	1.08 (4.00)	780.26 (153.86)	1.52 (4.18)	725.83 (162.13)	1.36 (4.45)	740.47 (124.01)	2.40 (4.90)
Neutral:								
Switch	739.40 (170.82)	8.29 (13.13)	872.67 (191.09)	6.16 (7.65)	752.27 (180.34)	5.93 (9.53)	804.11 (197.82)	7.15 (7.18)
Non-Switch	755.37 (160.76)	5.81 (10.67)	768.33 (157.91)	4.79 (6.98)	717.98 (160.54)	3.06 (7.39)	776.00 (156.15)	1.73 (3.98)

C.4 Discussion

This study asked whether long-term high-effort endurance exercise may protect against the age-related declines in tasks requiring attentional resources. We anticipated that supervets, defined as older adult volunteers with a 20+ year history of regular high-effort endurance exercise, would still have an advantage over our comparable group of volunteers not engaging in high-effort exercise.

Physiologically, while both groups had the same potential for fitness, our supervet group spent more time doing strenuous sports and muscle strength / endurance activities which resulted in them being more physically fit than our control group, as demonstrated by less percentage body fat and stronger hand-grip in males. There was also no difference in the two groups in terms of level of adherence to Mediterranean diet.

Cognitively, our analyses found no differences between supervets and controls in a speed of processing task (SRT), RT variability measures on the SRT, and visual search (Map TEA)[3]. On attention tasks, that required more processing resources and attentional control, subtle differences emerged.

In the Stroop-switch task at the initial time point supervets, unlike controls, did not show increased reaction times when switching from color-naming trials to word-naming trials. This lack of cost has not been seen before as it has been previously displayed in younger adults, older adults, and participants with MCI (Hutchison et al., 2010). Our supervets were able to transition between the two competing task requirements (color-naming and word-naming) without any cost. This suggests that long-term high-effort endurance exercise may have

[3] Multiple regression analyses using the cohort as a single continuous group and physical activity / physiological measures as predictors did not alter these outcomes.

improved mental flexibility, as measured in this attentional control task. This result confirms the results of Prakash et al. (2011) finding those with higher cardiorespiratory fitness having better performance in their hardest Stroop condition and Predovan et al.'s (2012) intervention, where their exercise group performed better in their incongruent switch condition. Alternatively supervets may have used the strategy of the Stroop-switch being a predictable task to elicit better performance (Coubard et al., 2011), while the controls did not.

At the follow-up time point however, the strategy utilized by supervets instead increased errors in color-naming trials relative to word-naming trials. This may reveal that the supervets' strategy, at least at follow-up, was actually an anticipation strategy and their attempt to be faster ended up resulting in more errors.

Supervets also displayed a differential strategy to controls on the RVIP task. Supervets started with faster reaction times and greater accuracy, but rapidly adjusted to a slower pace that they could maintain. Controls sustained a more paced reaction time from the start, with a shallower fatigue curve. Also in terms of accuracy, controls seemed to maintain the same level of accuracy until a significant drop towards the end. These results correspond well with reports from the sports psychology literature. Elite marathoners are reported to employ an "effort sense" allowing them to use a cognitive strategy to adjust their effort for relatively stable performance in their running (Morgan & Pollock, 1977). Here this effort sense was applied to the cognitive sustained attention task, eliciting adjustments in strategy.

The RVIP results may reflect greater metacognitive awareness, in conjunction with supervets being more motivated. Anecdotally, it was clear that the supervets were a highly motivated group who were much more keen to attend the 3 hour testing sessions and individually

committed to the view that their exercise regimen improved their cognitive health and wellbeing.

The subtle differences on more complex measures of attention converge to suggest that the supervets may be bringing different processing strategies to bear on these tasks. We suggest that these strategy differences are best accounted for by the motivational energy of the supervets. This is in line with previous studies reporting that motivational differences in participants elicit better performance on lab-based attention tasks (Deaton & Parasuraman, 1993; Tomporowski & Tinsley, 1996).

In contrast to the limited differences reported here from a comprehensive evaluation of cognitive performance in superveteran athletes, other recent studies of senior athletes (Tseng, Uh, et al., 2013; Winker et al., 2010); have reported significant benefits of exercise on cognition. Notably, however, they reported benefits in a relatively narrow range of executive function tasks amongst the many measures taken in each of these studies. Our study then is in line with these results as it is only in the most complex attentional tasks that we tested that we observed differences.

In conclusion, our evaluation of long-term high-effort endurance exercise in attentional tasks suggests only subtle differences relative to non-sedentary volunteers with a similar age, social, cognitive, diet and neuropsychiatric profile. These small differences manifest in tasks requiring application of processing resources, attentional control, and strategy and our results may indicate differences in motivation and metacognitive awareness between groups.

In future studies, the effect of motivation should be disentangled from the performance measures. This could be achieved by manipulating the motivation of participants during task performance or using an explicit index of individual motivation or effort in response to task.

Overall, the current results suggest that the benefits of long-term high-effort endurance exercise in older adults may not extend to substantive improvements in attention, when comparison is relative to non-sedentary participants similar in age, sex, IQ, education, diet, social, cognitive and neuropsychiatric profile.

Acknowledgments:

Thanks to David Bunce and Keith Hutchison for generously allowing us to use their tasks and guidance on implementation and analysis of these tasks.

D. **Article 3:** Are There Brain Structural Benefits of Long-Term High-Effort Endurance Exercise in Older Adults?

Abstract

Age-related decline in brain structure has been shown to be offset by increased exercise. However the results of long-term high-effort endurance exercise has been underexplored. In a cross-sectional design, we recruited older adults engaging in high-effort endurance exercise over at least twenty years, and compared their brain structure with a non-sedentary control group similar in age, sex, education, IQ, depression levels, and other lifestyle factors at two time points one year apart. We hypothesized that long-term high-effort endurance exercise would protect against the age-related decline in brain structure. Our findings, in contrast to previous studies, indicated that those participating in long-term high-effort endurance exercise, when compared without confounds to non-sedentary control volunteers, showed little substantive differences. Long-term exercisers only displayed higher diffuse white matter axial diffusivity, an index of axonal integrity, than controls, but this did not correlate with any cognitive differences.

Article III submitted to NeuroImage.

D.1 Introduction

D.1.1 The aging brain

Well-documented structural changes occur in the aging brain: cortical thinning and regional volumetric loss are observed (for reviews see Hedden & Gabrieli, 2004; Raz, 2000), and white matter integrity deteriorates (for reviews see Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009; Madden et al., 2012; Madden, Bennett, & Song, 2009).

Specifically for white matter integrity, it has been observed that mean diffusivity of water molecules within the white matter (MD) increases with age, while the fractional anisotropy of that diffusion (FA) decreases. Decline in FA is greater in anterior regions of the brain than in posterior regions (Madden et al., 2012). More recent studies have started looking more at the specific diffusivity measures, axial diffusivity (λ1) and radial diffusivity (RD). Axial diffusivity has been related to the myelin content of white matter tissue, while RD has been related to amount of demyelination and is proposed to be a marker of overall tissue integrity (Klawiter et al., 2011). However age-related differences in these parameters are not consistent and vary by brain region. In general, in white matter areas of decreased FA, RD increases in older adults, while λ1 does not change (Bennett et al., 2010; Burzynska et al., 2010; Zhang et al., 2010). However in a smaller proportion of separate white matter areas (Burzynska et al., 2010) or partially overlapping areas (Bennett et al., 2010), λ1 has been shown to decrease in older adults.

Volumetrically, in a typical study, Resnick, Pham, Kraut, Zonderman, & Davatzikos (2003) looking longitudinally at healthy older adults 59-85 observed significant decline in both grey and white matter volume from their first follow-up of 2 years even in a very healthy subgroup that did not develop any medical conditions or cognitive impairments during the 4-year period of evaluation.

These age-related volumetric changes differ by brain region (Salthouse, 2011). Uylings & de Brabander (2002) in their review observed the frontal cortex and hippocampal volume to especially decline with age, while Resnick et al. (2003) found that frontal and parietal lobe declined more than the other lobes. Looking longitudinally, Raz, Ghisletta, Rodrigue, Kennedy, & Lindenberger (2010) in middle-aged and older adults 49 years and older, observed significant volumetric decreases in the hippocampus, entorhinal cortex, orbital-frontal cortex, and cerebellum at 15 months. At 23 months, the authors began to observe significant volumetric decreases from baseline in caudate, prefrontal white matter, and the corpus collosum. In longitudinal studies, volumetric changes can be detected over as little as 4 months in both healthy younger (20-30) and older adults (60-70) in the hippocampus (Lövdén et al., 2012). Raz et al. (2013) in a longitudinal study of healthy younger (20-31) and older adults (65-80) over just 6 months, observed significant decline in volume of lateral prefrontal cortex, hippocampus, caudate nucleus, and cerebellum.

Individual differences in trajectory of these brain volume changes vary significantly though, as seen in both Raz et al. (2010) and Raz et al. (2013). Also many factors are likely to influence the rate of structural brain changes in older adulthood (Bickart et al., 2011; Kanai et al., 2012; Schinka et al., 2010). One such factor is exercise.

D.1.2 Exercise and the aging brain

Exercise may induce change in the aging brain or at least change the trajectory of decline (for reviews see Bherer, Erickson, & Liu-Ambrose, 2013; Hayes, Hayes, Cadden, & Verfaellie, 2013). Colcombe et al. (2003), using voxel-based morphometric (VBM) analysis techniques in older adults 55 years or older indeed observed deterioration in grey and white matter as a function of age, but the brain regions most affected by aging also showed the greatest benefit of fitness. In grey matter this was in the prefrontal, superior parietal, and temporal cortices. In white matter this was in the anterior tracts and transverse tracts running between frontal and

posterior parietal lobes. Colcombe et al. (2006) then did a 6-month aerobic exercise randomized control trial (RCT) with sedentary older adults 60-79 years with the control group doing stretching and toning non-aerobic exercises. Those in the aerobic exercise intervention group increased in aerobic fitness while those in the control group did not. The aerobic exercise intervention group showed increases in both grey and white matter volume over the period relative to the control group. Longitudinal VBM analysis found the largest increases to be in areas of the frontal lobes for both white and grey matter. Ruscheweyh et al. (2011) in an intervention with older adults 50-72 also observed correlations of increase in exercise with increase of the grey matter of prefrontal areas and the cingulate. Also Erickson et al. (2009) with older adults 59-81 years, looking exclusively at the hippocampus, observed that higher levels of fitness was associated with greater hippocampal volume and both of these were associated with better spatial memory performance. They also observed that hippocampal volume partially mediated the relationship between higher fitness levels and better spatial memory. Therefore the authors concluded that higher levels of aerobic fitness were correlated with higher hippocampal volumes which enhanced spatial memory function. Following on, Erickson et al. (2011) then reported an RCT with older adults 55-80 years, observing that a 1-year aerobic exercise intervention increased hippocampal volume, while in their stretching control group hippocampal volume decreased. Greater increases in fitness were correlated with greater increases in hippocampal volume, and there was a positive relationship between higher aerobic fitness levels and spatial memory and between increased hippocampal volume and improved spatial memory. Also the study interestingly found that while hippocampal volume declined in the stretching group, higher fitness before the intervention was protective against volume loss and partially attenuated the decline in volume.

Recent research has explored longer-term benefits of exercise, as revealed by studying older athletes. Most recently Tseng, Uh, et al. (2013) reported a cross-sectional study that compared a small group of twelve older runners with sedentary older adults also similar in age, sex, and

education. VBM measures of brain volume indicated that their older athletes had more grey matter concentrations in right parietal lobe, cuneus, and the culmen of the cerebellum and more white matter concentrations in precuneus, subgyral occipital lobe, and inferior temporal subgyral temporal lobe. Their runners also did better than sedentary older adults in executive function tasks of category fluency and letter fluency. In a similar cohort comparing ten older runners with twelve sedentary older adults, Tseng, Gundapuneedi, et al. (2013) reported their runners had better white matter integrity, as indexed by TBSS (Tract-based spatial statistics, S. M. Smith et al. 2006). In fractional anisotropy (FA) and mean diffusivity (MD) measures, they observed differences in brain regions associated with motor function; front-and-back connections related to visuospatial function, motor control and coordination; and regions associated with memory function. They also observed that runners had lower deep white matter hyperintensity volume. From this evidence, the authors suggested that long-term exercise may preserve white matter integrity from age-related changes. While participants in these older athlete studies were similar in age, sex, and education level, but their cross-sectional design meant that there was no way to unequivocally attribute the observed differences to the exercise regimen of the volunteers.

D.1.3 Exercise and cognitive aging

Looking just at cognition, exercise may protect against age-related change. This has been evidenced in many studies including retrospective and short to medium and long term studies, where increased exercise or fitness has been shown to confer cognitive benefits (Barnes et al., 2003; Chang et al., 2012; Colcombe & Kramer, 2003; Etnier et al., 1997; Hindin & Zelinski, 2012; Middleton et al., 2010; P. J. Smith et al., 2010; Weuve et al., 2004). Systematic reviews of studies involving aerobic exercise interventions, from 2 months to 6 years duration, also concluded that exercise improves cognitive ability, especially executive function, in normal healthy older populations (Angevaren et al., 2008; Chang et al., 2012; Colcombe & Kramer,

2003; Etnier et al., 1997; Hindin & Zelinski, 2012). Smith et al. (2010) in their meta-analytic review of RCTs of limited duration interventions found that exercise improved attention and processing speed, executive function, and memory in both healthy older populations and in older people with mild cognitive impairment.

Many of the aforementioned MRI and behavioral studies though either were interventions with sedentary volunteers or used sedentary volunteers as their control group. Also the cross-sectional studies compared groups that were not expressly similar on measures of lifestyle, age, gender, and depression, all of which complicate interpretation of the data.

D.1.4 Our study

The current study engaged two older adult populations that were distinctly different in their exercise profiles. The first group, super veteran athletes ("supervets"), comprised individuals whose age (60+) qualified them for super veteran categorization by UK Athletics, and who had engaged in long-term (minimum 20 years) high effort endurance exercise. The second group, non-sedentary control volunteers, was similar in age and full-time education to the supervet group, socially active, but not exercising beyond regular levels. With these populations we set out to test the effects of long-term high effort endurance exercise on brain structure in older adults. Our groups were similar in age, sex, education level, intelligence, depression level, social network, cognitive activities, adherence to Mediterranean diet, and potential for physical fitness. More importantly, we report longitudinal measures of performance and brain structural changes over a 12-month period, so that in our study we can establish not only whether there are differences between the groups but also whether age-related changes over 12 months differentiate the groups. We hypothesized that our supervet group when compared to this non-sedentary socially active control group would still show benefits to brain structure from their long-term exercise regime.

D.2 Methods

D.2.1 Ethics Statement

Ethical approval was obtained from the University of Sussex Life Sciences & Psychology Cluster based Research Ethics Committee and the Brighton and Sussex Medical School Research Governance & Ethics Committee. Written consent was obtained from all participants.

D.2.2 Participants

For inclusion in the study, we chose non-smoker (never smoked or not smoked in the past 5 years) participants aged 60-85, on stable medication if any during the past 12 months, with English proficiency equivalent to that of a native speaker.

For our supervet group we required active exercise at a high level mostly via running, swimming, and/or cycling (self-paced sports) for the past 20 or more years. We chose only self-paced sports because athletes of interceptive-dominant sports have been shown to have faster reaction times (Voss et al., 2010b) which may affect cognitive performance. Also we chose high effort endurance exercise to differentiate from exercise consisting of just short high-effort bursts or sustained low effort.

We excluded participants if they had a history of stroke, myocardial infarction, recently diagnosed diabetes, very high blood pressure (systolic above 200 and diastolic above 100), psychiatric or neurological disorders (self-reported), or were suffering from clinical depression. We also excluded people with pacemakers, metal in their eyes, or with any history of concussion from head injury lasting more than a few seconds.

From a larger sample recruited for behavioural testing only, we recruited 15 supervets and 14 controls; at the initial time point, and 15 supervets and 12 controls returned at 1-year follow-up. At follow-up one control moved too much during the MPRAGE scan rendering the data unusable.

D.2.3 Procedure

MRI techniques

Diffusion Tensor Imaging (DTI)

DTI quantifies the magnitude and direction of diffusion of water in tissue and is sensitive to pathology (Tofts et al., 2003) and to age-related changes (Charlton et al., 2006; Tseng, Gundapuneedi, et al., 2013).

DTI data in the MRI is acquired with diffusion weighting along six or more orientations, which are then used to calculate the diffusion tensor, the simplest representative diffusion model. The diffusion tensor has three orthogonal eigenvectors (e_1, e_2, and e_3), representing the principle axes of the tensor and expressing the direction of diffusion. The axes are scaled by the extent of diffusion along that direction represented by the eigenvalues of the diffusion tensor (λ_1, λ_2, and λ_3), expressing the magnitude, or speed of movement, of the diffusion. See Figure D.1 for a schematic representation.

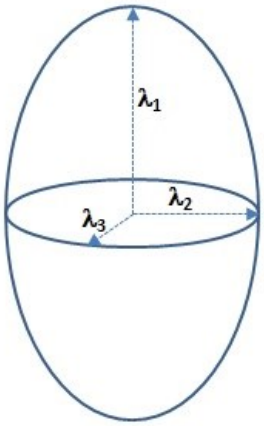

Figure D.1. Schematic representation of the diffusion tensor. The arrows represent the orientation of the three orthogonal vectors. The axes are scaled by the eigenvalues, λ_1, λ_2, and λ_3.

Mean diffusivity (MD), also known as the Apparent Diffusion Coefficient (ADC), is a measure of the extent of diffusion in each voxel and is calculated as the average of the 3 eigenvalues of the diffusion tensor, $\lambda_{1\text{-}3}$. MD is high in environments where molecules can move in any direction easily, such as in cerebral spinal fluid. Cell walls, nerve fibres, and other biological barriers in the brain impede the free diffusion of water molecules and therefore reduce measures of MD. As these biological barriers break down, MD increases (Tofts et al., 2003). For more specific measures of diffusivity, MD can be further decomposed into λ1 and radial diffusivity.

λ1, the principal diffusion direction, is assumed to be parallel to neural fibers in the direction of the axon and so is called axial diffusivity or parallel diffusivity. Decreases in axial diffusivity have been previously observed in axonal damage due to axonal swelling and Wallerian degeneration (Song et al., 2003). It has been related to the amount of myelin content as observed in an ex-vivo DTI and histochemical staining study (Klawiter et al., 2011). Radial diffusivity (RD) or perpendicular diffusivity is the average of λ2 and λ3, therefore (λ2 + λ3)/2. It has been used as an assessment of the degree of restriction due to membranes and other effects and is sensitive to pathology in some conditions (Vaillancourt et al., 2009). It has been related to amount of demyelination and it has been proposed to be a marker of overall tissue integrity (Klawiter et al., 2011). Looking at white matter regions, recent studies have observed in some regions RD to increase and λ1 decreased with age. In other white matter regions, it was observed that λ1 decreased but RD did not change with age (Bennett et al., 2010; Burzynska et al., 2010).

Fractional anisotropy (FA) measures the directionality of diffusion, that is, how restricted to a particular direction the water molecules are diffusing. In white matter tracts, diffusion is more restricted to occur along the neural fiber than across it; therefore white matter tracts have higher FA. In contrast, grey matter, where diffusion is not restricted in any particular direction,

has lower FA. If biological barriers in the brain are destroyed, then FA decreases in directional fibers (Tofts et al., 2003). Rovaris et al. (2003) concluded that FA provides an independent index of white matter microstructure.

Histogram analysis:

Histogram analysis is sensitive to subtle diffuse disease (Tofts et al., 2003) and aging (Charlton et al., 2006). For example, comparing patients with multiple sclerosis (MS) to controls without MS, Cercignani, Bozzali, Iannucci, Comi, & Filippi (2001) observed diffuse tissue damage in the white matter of patients with MS, in the form of increased overall MD values. Looking at aging, Charlton et al. (2006) observed cross-sectionally middle-aged and older adults and found progressive reduction in FA and increase in MD with age, which correlated with declines in cognitive measures.

Histograms are constructed by calculating the number of voxels or "voxel count" within an image volume whose values lie within a number of discrete ranges or "bins" for a given MR parameter. In this study we constructed an FA histogram with a total range of 0.01 to 1.01 with 200 bins, resulting with a bin width of 0.01. For MD, λ1, and RD we constructed histograms with a total range of 20 x 10^{-6} mm^2/s to 3500 x 10^{-6} mm^2/s with 174 bins, resulting with bin widths of 20 x 10^{-6} mm^2/s.

To account for differences in head size, we normalized all histograms to sum to 20, using the following equation:

$$\sum\left(\frac{number\ of\ voxels\ per\ bin}{total\ number\ of\ voxels}\ x\ 20\right) = 20$$

Each bin would then show the relative distribution of voxels for each given MR parameter. Histograms are often noisy, especially around the peak, making it more difficult to automatically extract informative parameters such as the histogram maximum (peak height,

PH) and the histogram mode (peak position, PP). A solution is to use a Gaussian smoothing kernels on the histogram. The widths of the smoothing kernels were chosen to be similar to the bin widths because they smoothed without significantly reducing the true peak height. An example of an unsmoothed and smoothed histogram is shown in Figure D.2.

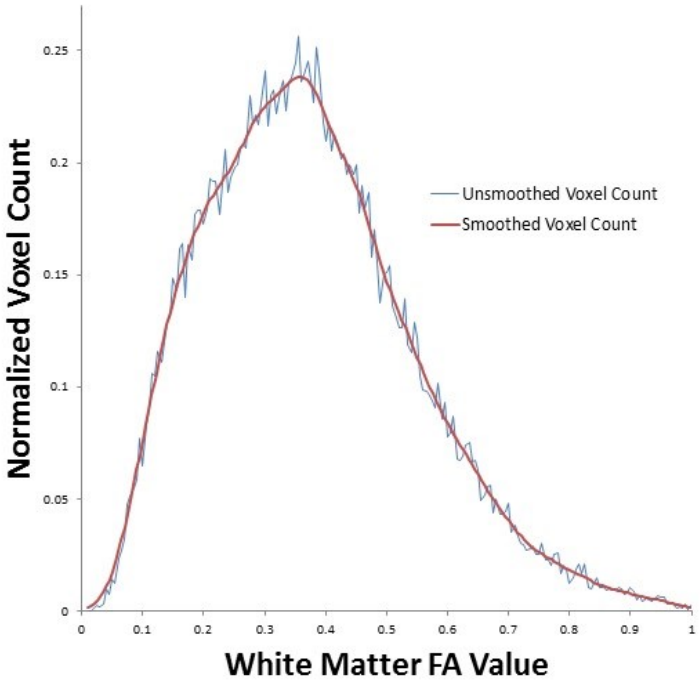

Figure D.2. The distribution of normalized voxel counts of FA in White Matter, unsmoothed (thin line) and smoothed (thick line).

We summarize the important information in our histograms by calculating the summary indices of PH, PP, and mean (Tofts et al., 2003). See Figure 3 for histogram with PH and PP labeled. PH may be used as a measure of tissue damage: if the tissue takes on a wider range of parameter values, its histogram will be broader and the normalized peak height will be reduced. This has been observed in many studies of MS, for example Cercignani et al. (2001). In contrast, PP may be used to identify if subtle global shifts occur in the tissue, for example again in MS there are shifts in both grey and white matter in FA and MD have been observed (Vrenken et al., 2006). The histogram can be further characterized by the mean value, which

has been used in many past histogram studies (for example: Fushimi et al., 2007; Holtmannspötter et al., 2005; Rashid et al., 2004).

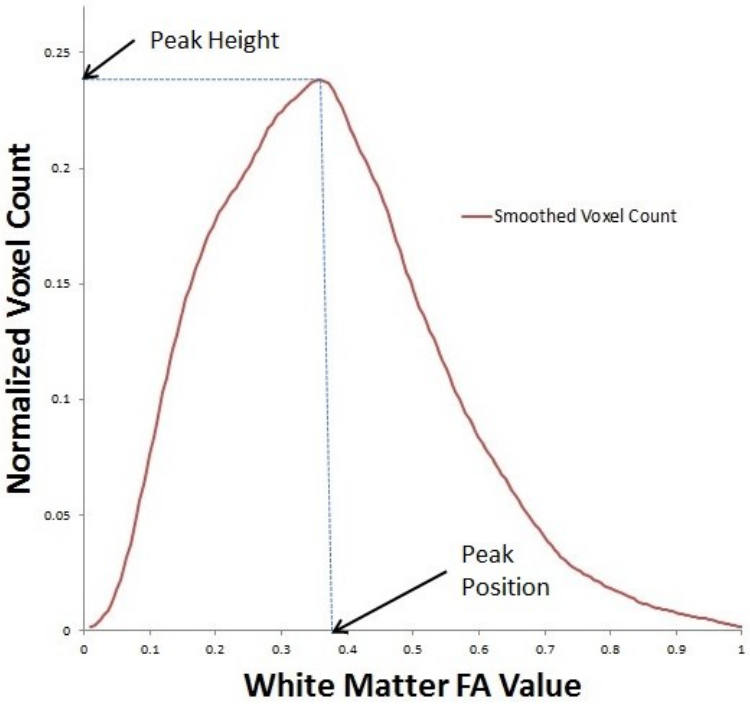

Figure D.3. Smoothed normalized histogram of FA in White Matter with Peak Height and Peak Position labeled.

However histogram analysis is not sensitive to localized changes since our ROIs were over the whole brain by tissue type. For isolating more localized changes we used TBSS. TBSS is a voxel –wise analysis that looks at group differences isolated to the white matter tracts.

Data collection

Participants all took part in the study at the behavioral testing facilities in the School of Psychology at the University of Sussex and MRI sessions at the Clinical Imaging and Sciences Centre (CISC) at the Brighton and Sussex Medical School. Follow-up MRI sessions took place approximately 12 months later. Cognitive data was obtained from separate parallel behavioral sessions that took place 12 months apart also. Time intervals in relation to MRI and cognitive

sessions varied per participant, but were kept as closely linked as possible, allowing for the individual availability of the volunteers.

Participants were consented by a researcher on each occasion. The cognitive testing sessions lasted approximately 3 hours; including breaks as and when volunteers wanted. Prior to the imaging session, volunteers underwent a MRI safety screening by a radiographer. They then took part in an MRI scan session of less than 30 minutes. Participants were compensated for their transportation costs and parking.

Cognitive test materials

Cognitive tasks are described in more detail in previous papers (Article I and Article II). In brief we utilized speed of processing tasks; executive function tasks; memory tasks: episodic memory, working memory, and prospective memory; and attention tasks: visual search, sustained attention, and attentional control.

MRI protocol

All images were acquired on a Siemens 1.5 Tesla Avanto MRI scanner (Siemens, Erlangen, Germany). High-resolution anatomical images were acquired using a three-dimensional T1-weighted magnetisation prepared rapid acquisition gradient echo (MPRAGE) sequence [TR = 1160 ms; TE= 4.44 ms; inversion recovery time (TI) = 600 ms; field of view (FOV), 230 x 230 mm^2; matrix size, 256 x 256; flip angle θ = 15 degrees; voxel dimensions, 0.9 x 0.9 x 0.9 mm^3; acquisition time, 5 min].

Diffusion-weighted images were acquired using an echo planar imaging sequence [TR = 12.4 s; TE = 111 ms; echo spacing, 0.83 ms; FOV, 240 x 240 mm^2; matrix size, 96 x 96; voxel dimensions, 2.5 x 2.5 x 2.5 mm^3; acquisition time, 7 min]. Diffusion gradients were applied

along 30 noncollinear directions (b_{max} = 1000 s/mm^2). A nondiffusion-weighted (b ~ 0) volume was also acquired.

Image post-processing:

DTI

Histogram volumetric analysis:

DTI data were pre-processed using the FSL suite (S. M. Smith et al., 2004; Woolrich et al., 2009). DTI volumes were realigned using the eddy current correction module. Then they were skull-stripped using the brain extraction tool and segmented into white matter and grey matter region of interests (ROIs) using the New Segment tool in SPM (Statistical Parametric Mapping, Functional Imaging Laboratory, University College London). The diffusion tensor was calculated for each ROI using DTIfit, yielding an FA map, a T2-weighted image free from diffusion weighting (S0), the 3 eigenvalues of the diffusion tensor λ1-3, and an MD map. RD was calculated as described above.

For each MR parameter we plotted the frequency distribution normalized to 20 units. The resulting histograms were smoothed using a gaussian kernel, for FA the kernel widths were 0.009 and 0.02 for grey matter and white matter respectively; for MD, λ1, and RD the kernel widths were 20 x 10^{-6} mm^2/s and 10 x 10^{-6} mm^2/s for grey matter and white matter respectively. We then calculated peak height (PH), peak position (PP), and mean for each histogram per ROI per DTI parameter.

MR parameter indices (PH, PP, Mean) were compared at each time point using ANCOVAs between groups (supervet vs. control), $p < .05$, with age and sex (Hsu et al., 2008; Kanaan et al., 2012) as covariates.

Tract-based Spatial Statistics (TBSS):

Tract-based spatial statistics (TBSS) (S. M. Smith et al., 2006), part of FSL, was performed on DTI data. All individual FA maps were normalized to the standard Montreal Neurological Institute (MNI) 152 FA space template by first finding the most representative participant brain as a target which is then affine-aligned into MNI152 space. Each participant is then nonlinear transformed to the target FA image and affine transformed from target to MNI space. A mean across the groups was generated, and a tract mask or "skeleton" was computed representing the tract centers common to both groups. Individual FA maps were projected onto this skeleton in order to modify or "individualize" the tract pathways. This process involves the identification of voxels (located close to the tract skeleton) that have higher FA values than those masked by the original skeleton. If higher values are found, they are substituted for those masked by the skeleton. Inference testing was then carried out. Non-FA images of MD, λ1, and RD were warped to the representative participant brain and then projected to individualized FA "skeletons" before inference testing was carried out.

TBSS inference testing was employed to identify differences between supervet and control groups. As the null distribution was not known, a permutation-based inference method was employed (Nichols & Holmes, 2002). A two-sample nonparametric test was carried out with 10000 permutations using a threshold-free cluster enhancement (TFCE) method. In contrast with other such techniques, this method has the advantage that no arbitrary initial cluster-forming threshold needs to be selected. The computed cluster is tested against a critical cluster size: if the resulting cluster is larger than the critical size, the cluster is significant.

Anatomical

Whole brain volume analysis:

MPRAGE volumes were segmented into white matter (WM), grey matter (GM), and cerebral spinal fluid (CSF) ROIs. This was added together to get the intracranial volume (ICV). Whole brain (WB) volume was calculated by adding WM and GM. Then each ROI – WM, GM, WB –

was divided by the ICV to calculate the percentage of the whole volume each ROI took. All data was then compared using two-tailed t-tests between groups, $p < .05$ at the initial time point. When including the follow-up time point, mixed ANCOVAs were used with time point as a repeated measures factor, groups as an independent measures factor, and age and sex (Good et al., 2001) as covariates.

Voxel-based morphometry (VBM):

For initial time point VBM, we used Christian Gaser's VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) for the SPM8 package (Wellcome Department of Cognitive Neurology, London, UK (J Ashburner & Friston, 2000)) in Matlab (MathWorks, Natick, MA, USA). We skull stripped and warped T1-weighted anatomical images into MNI space and segmented GM, WM, and CSF within a unified segmentation model (John Ashburner & Friston, 2005). Modulated normalized segmentations of GM and WM were then smoothed using an 8x8x8-mm^3 kernel. Two-sample t-tests were defined to detect significant group volume differences. The family-wise error correction was carried out at $p < .05$.

Including follow-up, we used VBM longitudinal analysis in VBM8. Follow-up participants' volumes were non-linearly registered to their own initial time point volumes and spatial normalization is estimated for initial time point volumes only and applied to all time points. Mixed ANOVAs were defined to detect group volume differences, time point volume differences, and interactions between the two. The family-wise error correction was carried out at $p < .05$.

Freesurfer volume and cortical thickness:

Both time points were automatically processed using the longitudinal stream in Freesurfer (Reuter, Schmansky, Rosas, & Fischl, 2012). For each participant across both time points an unbiased within-subject template space and image (Reuter & Fischl, 2011) was created using

robust, inverse consistent registration (Reuter et al., 2012). Then several processing steps, including skull stripping, Talairach transforms, atlas registration as well as spherical surface maps and parcellations, were initialized across both time points using common information from the within-subject template, which significantly increases reliability and statistical power (Reuter et al., 2012).

For volume and cortical thickness analyses we looked specifically at areas most previously seen to be affected by aging and/or were previously shown to be different for those that exercised. For volumes we chose hippocampus (Erickson et al., 2009, 2011; Lövdén et al., 2012; N. Raz et al., 2010; Uylings & de Brabander, 2002), cerebellar cortex (N. Raz et al., 2013, 2010; Tseng, Uh, et al., 2013), cerebellar white matter (N. Raz et al., 2013, 2010; Tseng, Uh, et al., 2013), and caudate (N. Raz et al., 2013, 2010). For cortical thickness we looked at entorhinal cortex (N. Raz et al., 2010), frontal lobes (Colcombe et al., 2003; Colcombe, Erickson, Scalf, Kim, Prakash, McAuley, et al., 2006; N. Raz et al., 2013; Resnick et al., 2003; Uylings & de Brabander, 2002), and parietal lobes (Colcombe et al., 2003; Resnick et al., 2003; Tseng, Uh, et al., 2013).

Volumes for each hemisphere across subjects and between time points were extracted and divided by ICV; age and sex were used as covariates and groups were compared using ANCOVAs at the initial time point, $p < .05$. For the follow-up time point mixed ANCOVAs were used with time point as a repeated measures factor, group as an independent measures factor, and age and sex as covariates.

Initial time point cortical thickness and cortical thickness symmetrized percent change (SPC), which divides the rate of change by average thickness over the two points, giving more statistical power than percent change relative to time point 1 thickness (since average thickness is less noisy than thickness at time point 1), were extracted. At the initial time point, age and sex were used as covariates, and groups were compared using ANCOVAs, $p < .05$. With

the follow-up cortical thickness SPC, mixed ANCOVAs were used with time point as a repeated measures factor, group as an independent measures factor, and age and sex as covariates.

Correlations with cognitive data

The outcomes from the cognitive testing are reported in full detail in previous papers (Article I and Article II). For the purposes of these analyses, correlations were computed between group differences found in cognitive data and group differences in MRI data.

Technical issues:

Between the initial time point and 1-year follow-up MRI sessions the MRI scanner gradients were upgraded yielding an increase in gradient amplitude (from 33mT/m to 44 mT/m) and slew rates.

For our DTI measures we ensured diffusion gradient amplitudes were identical for both time points, but differences remained in the imaging gradients. As a result, the DTI data between sessions were not directly comparable. Daily independent QA data using the EPI sequence also showed there were no SNR step changes as a result of the upgrade.

Any potential differences in T1-weighted MPRAGE scans as a result of the hardware upgrade were accounted for in analyses: participants' volumes at each time point were normalized to templates (Freesurfer longitudinal); normalized to initial time point volumes (VBM longitudinal), or intracranial volumes at each time point were used to normalize the data as is standard practice.

D.3 Results

D.3.1 Characteristics

There were no differences between supervet and control groups in sex, age, years of education, and IQ (Table D.1).

Table D.1

Means, Standard Deviations, and Significances between Supervet and Control Group Characteristics

Measure	Supervets Mean (SD)	Controls Mean (SD)	Significance
Characteristics:			
Sex[a]	4 female	5 female	$p = .599$
Age (years)	68.93 (5.18)	67.57 (6.45)	$p = .535$
Education (years)[b]	16.20 (2.81)	15.79 (3.09)	$p = .708$
IQ[c]	117.92 (4.58)	119.76 (5.34)	$p = .350$‡

Note: a. Chi-squared test used. b. Years of full-time education. c. Full Scale Pre-morbid Intelligence Quotient derived from National Adult Reading Test. ‡ - From 1000-sample bootstrap results.

There were no differences between groups at either time points in level of depression, Mediterranean diet (only analyzed initial time point), lung function measures, social network, and cognitive activities (Table D.2).

Table D.2

Means, Standard Deviations, and Significances between Supervet and Control Groups Depression, Diet, Lung Function, and Social and Cognitive Characteristics

Measure	Supervets Mean (SD)	Controls Mean (SD)	Significance at T0	Supervets Mean (SD)	Controls Mean (SD)	Significance at T1
Depression:						
Depression[a]	0.29 (0.61)	1.07 (1.77)	$p = .150$‡	0.47 (0.64)	0.83 (1.59)	$p = .497$‡
Diet:						
Mediterranean Diet Score	4.73 (1.33)	4.36 (2.02)	$p = .572$‡			
Lung Function:						
Forced Expiratory Volume 1st Second (L)	2.83 (0.81)	2.91 (0.75)	$p = .809$	3.05 (0.56)	2.96 (0.71)	$p = .727$
Forced Vital Capacity (L)	3.86 (0.92)	3.52 (0.98)	$p = .374$	3.97 (0.64)	3.57 (0.80)	$p = .201$
Forced Expiratory Ratio (%)	76.92 (83.25)	83.25 (9.04)	$p = .137$	76.54 (7.61)	82.40 (8.87)	$p = .103$
Peak Expiratory Flow (L/min)	417.79 (139.19)	439.08 (151.03)	$p = .712$	485.31 (110.58)	460.90 (130.83)	$p = .633$
Social and Cognitive Activities:						
Social Network[b]	18.80 (4.75)	18.36 (5.72)	$p = .822$	20.40 (4.79)	17.92 (4.96)	$p = .188$‡
Cognitive Activities[c]	51.73 (6.18)	51.25 (8.04)	$p = .858$	49.60 (8.24)	52.18 (7.78)	$p = .416$

Note: a. From Geriatric Depression Scale score. a. Physical Activity Scale for the Elderly score. b. From Lubben Social Network Scale. c. From Florida Cognitive Activity Scale. ‡ - From 1000-sample bootstrap results.

D.3.2 Physical Activity and Physiological Indices of Fitness

Supervets trended towards taking part in more physical activities overall than controls at the initial time point and took part in more physical activities overall at follow-up. Interrogating further, supervets took part in more strenuous sports than controls at both time points; they did not differ at either time point in other types of leisure activities (Table 3, Physical Activity). At the follow-up time point we evaluated lifetime physical activity maintenance for each leisure activity category, and in these subgroups, supervets (M = 97.33%, SD = 7.04) maintained a higher percentage of their leisure activities when compared to controls (M = 74.23%, SD = 18.21) M_{diff} = 23.10%, 95% CI [12.09, 34.97], p = .008.

In terms of physiological measures in these subgroups, male supervets only trended towards having less percentage fat than male controls at the initial time point and only female supervets had significantly less percentage fat than female controls at the follow-up time point. Also only male supervets trended towards having more hand-grip strength than male controls at the initial time point (Table D.3, Physiological).

Table D.3

Means, Standard Deviations, and Significances between Supervet and Control Groups Physical Activities and Physiological Characteristics

Measure	Supervets Mean (SD)	Controls Mean (SD)	Significance at T0	Supervets Mean (SD)	Controls Mean (SD)	Significance at T1
Physical Activity:						
PASE[a]	222.88 (60.50)	176.87 (78.42)	$p = .086$	230.69 (60.51)	167.56 (57.54))	$p = .011$*
Strenuous Sports (average time per day, hours)	1.28 (0.91)	0.14 (0.34)	$p = .003$‡*	1.06 (0.60)	0.13 (0.17)	$p = .001$‡*
Physiological:						
Body Fat Males (%)	22.19 (4.50)	26.19 (4.74)	$p = .079$‡	24.94 (4.71)	27.94 (4.83)	$p = .192$
Body Fat Females (%)	32.15 (5.13)	40.62 (8.04)	$p = .112$	34.85 (3.56)	42.58 (3.43)	$p = .020$*
Hand-grip Strength Male (kg)	41.23 (4.12)	36.33 (5.13)	$p = .052$	42.19 (4.80)	40.43 (5.15)	$p = .464$
Hand-grip Strength Female (kg)	28.40 (4.17)	25.18 (3.82))	$p = .266$	28.18 (3.76)	26.53 (4.50)	$p = .620$

Note: a. Physical Activity Scale for the Elderly score. * - $p < .05$. ‡ - From 1000-sample bootstrap results.

D.3.3 MRI data

Initial time point:

At the initial time point there were no significant differences between groups for any of our analyses: DTI histogram, *ps* > .05; TBSS; Whole Brain Volume in GM, WM, and WB, *Fs* < 1; and VBM.

Freesurfer Volume

For hippocampus, there were no differences in percentage volume between groups in the left hemisphere, $F(1, 22) = 1.64$, $p = .213$, or right hemisphere, $F(1, 22) = 2.01$, $p = .171$. For cerebellar white matter, there were no differences between groups in left hemisphere, $F(1, 22) = 1.71$, $p = .203$, or right hemisphere, $F < 1$. For cerebellar cortex, there was no difference between groups in left hemisphere, $F(1, 22) = 3.01$, $p = .097$, and a trend in right hemisphere, $F(1, 22) = 3.83$, $p = .063$: relative to controls ($M = 2.98\%$, $SD = 0.58$), supervets had a higher percentage volume ($M = 3.39\%$, $SD = 0.35$). For caudate, there were no differences between groups in left hemisphere or right hemisphere, $Fs < 1$.

Freesurfer Cortical Thickness

For the entorhinal cortex, there were no differences in average cortical thickness between groups the left hemisphere or right hemisphere, $Fs < 1$. For the frontal lobe there were no differences between groups in left hemisphere or right hemisphere, $Fs < 1$. For the parietal lobe, there was a difference in left hemisphere, $F(1, 22) = 4.62$, $p = .043$: relative to supervets ($M = 2.25$ mm, $SD = 0.08$), controls had greater average thickness ($M = 2.32$ mm, $SD = 0.07$); there was no difference between groups in right hemisphere, $F(1, 22) = 2.41$, $p = .135$.

The observed difference in left parietal lobe did not survive the correction for multiple comparisons.

Follow-up time point:

DTI

Supervets ($M = 1004.00 \times 10^{-6}$ mm^2/s, $SD = 33.12$) had significantly higher white matter axial diffusivity (λ1) than controls ($M = 971.67 \times 10^{-6}$ mm^2/s, $SD = 28.87$), $F(1, 23) = 5.96$, $p = .023$. There were no other differences between groups for any of the other indices in all parameters.

No differences were found between groups using TBSS, meaning these differences were not localized to any specific white matter tracts.

Anatomical

Whole Volume Analysis:

For grey matter percentage, there were no differences between groups, $F < 1$, between time points, $F(1, 22) = 1.68$, $p = .208$, nor was there a group by time point interaction, $F < 1$.

For white matter percentage, there were no significant differences between groups or between time points, $Fs < 1$. There was a group by time point interaction trend, $F(1, 22) = 4.14$, $p = .054$: relative to supervets ($M = 0.03\%$, $SD = 0.14$) controls decreased in percentage volume ($M = -0.09\%$, $SD = 0.11$).

This interaction trend was reflected in the whole brain analysis. Whole brain volume percentage trended towards decreasing from the initial time point to follow-up, $F(1, 22) = 3.43$, $p = .077$, qualified by a group by time point interaction trend, $F(1, 22) = 3.98$, $p = .058$: relative to supervets ($M = -0.02\%$, $SD = 0.10$) controls decreased in whole brain volume ($M = 0.17\%$, $SD = 0.21$).

Correcting for multiple comparisons, no significant effects remained.

VBM Analysis:

There were no significant differences between groups or time point and no significant interactions between the two.

Freesurfer Volume:

Including follow-up, for left hippocampal percentage volume there were no significant differences between groups, $F(1, 22) = 2.69$, $p = .116$, between time points, $F(1, 22) = 2.98$, p

= .098, nor a group by time point interaction, $F(1, 22) = 1.67$, $p = .209$. For right hippocampal percentage volume there was no significant difference between groups, $F(1, 22) = 1.58$, $p = .222$, but there was a significant effect of time point $F(1, 22) = 5.29$, $p = .031$: both groups decreased in volume from the initial time point ($M = 0.28$ %ICV, $SD = 0.03$) to follow-up ($M = 0.27$ %ICV, $SD = 0.03$), there was no group by time point interaction, $F(1, 22) = 2.24$, $p = .149$.

For left cerebellar white matter percentage volume there were no significant differences between groups, $F(1, 22) = 1.98$, $p = .179$, between time points, $F < 1$, nor a group by time point interaction, $F < 1$. For right cerebellar white matter there were no significant differences between groups, between time points, nor a group by time point interaction $Fs < 1$.

For left cerebellar cortex percentage volume there were no significant differences between groups, $F(1, 22) = 2.20$, $p = .152$, between time points, $F < 1$, but there was a group by time point interaction, $F(1, 22) = 5.49$, $p = .032$: relative to supervets ($M = -0.04$%, $SD = 0.006$) controls increased in percentage volume ($M = 0.09$%, $SD = 0.17$). For right cerebellar cortex there were no significant differences between groups, $F(1, 22) = 2.99$, $p = .098$, between time points, $F < 1$, but there was a group by time point interaction, $F(1, 22) = 4.31$, $p = .050$: relative to supervets ($M = -0.04$ %ICV, $SD = 0.07$) controls increased in percentage volume ($M = 0.09$, $SD = 0.21$).

For left caudate percentage volume there were no significant differences between groups, between time points, nor a group by time point interaction, $Fs < 1$. For right caudate there was no significant differences between groups, between time points, nor was there a group by time point interaction, $Fs < 1$.

When correcting for multiple comparisons, no significant effects remained.

<u>Freesurfer Cortical Thickness:</u>

For entorhinal cortex thickness symmetrized percent change, there was not a significant difference between groups in either left hemisphere or right hemisphere, *Fs* < 1. There was a significant difference between groups in frontal lobe cortical thickness SPC in left hemisphere, $F(1, 22) = 6.75$, $p = .016$: relative to supervets ($M = -0.15\%$, $SD = 1.29$), controls increased in thickness ($M = 1.43\%$, $SD = 1.36$); but only a trend in right hemisphere, $F(1, 22) = 3.63$, $p = .070$: relative to supervets ($M = -0.12\%$, $SD = 1.75$), controls increased in thickness ($M = 1.45$, $SD = 1.48$). There were no significant differences between groups in parietal lobe in both left hemisphere, $F < 1$, and right hemisphere, $F(1, 22) = 1.44$, $p = .243$.

Correcting for multiple comparisons, no significant effects remained.

Correlations with Cognitive Data

A full set of the cognitive data analyses have been reported elsewhere (Article I and Article II). In order to consider whether structural differences are related to cognitive differences previously observed, we computed a fixed number of correlations, on cognitive measures where differences were recorded between the groups. These were: Simple Reaction Time, Reaction Time cost in a PM task, and Switch RT in a modified Stroop task.[4]

For MRI measures we entered follow-up White Matter Axial Diffusivity, percentage white matter change, and percentage whole brain change.

Using these and correcting for multiple comparisons we found no correlations between any cognitive and MRI measure entered.

D.4 Discussion

In this study, we explored brain structural differences between superveteran athletes, engaging in long-term high-effort endurance exercise, and non-sedentary control volunteers;

[4] PM task was a Focal PM task, switch reaction time was from the Word-Neutral condition, which was the easiest switch trial.

and we explored potential differences in age-related change in brain structure at 12 month follow-up. Our study accounted for many factors by using groups that were statistically similar in age, sex, education, IQ, depression levels, adherence to Mediterranean diet, and potential for fitness.

Similar to our cognitive data (described in Articles I and II), our MRI data exposed very few differences between groups. Only one difference was statistically significant: supervets showed higher white matter axial diffusivity than controls at the follow-up time point. It is interesting that using the traditional parameter of MD there was no difference between groups, but when looking specifically at the parallel and perpendicular diffusivity measures of axial and radial diffusivity respectively, we saw diffuse differences in favor of higher axial diffusivity across the white matter in the supervets. Differences in this MR parameter are most likely related to myelin content (Klawiter et al., 2011), in turn interpreted as greater neural integrity (Madden et al., 2009; Zhang et al., 2007). If aging decreases axial diffusivity in many white matter regions (Bennett et al., 2010; Burzynska et al., 2010), then the higher diffusivity in supervets would suggest they are showing less age-related decline. Without any localized changes though, this finding is hard to interpret (Wheeler-Kingshott & Cercignani, 2009). Regardless, any consequence of this difference for cognitive performance was not apparent in our correlation analysis.

It is possible that this higher axial diffusivity in supervets may indicate a higher brain reserve capacity that is not apparent now in cognitive tasks but may be beneficial in the longer term by retarding age or disease-related cognitive decline (Tucker & Stern, 2011).

In terms of hippocampal volume, we did not observe higher volumes in supervets compared to controls at the initial time point nor were there significant hippocampal volume changes over the 1-year period differentiating the groups. This is in contrast to the results reported by Erickson et al. (2009, 2011) when looking at amount of physical activity and for their acute

exercise intervention in older adults. We did not observe any differences in our VBM analyses, in contrast to Colcombe et al. (2003) and Tseng, Uh, et al. (2013), nor did we observe differences in VBM longitudinal analyses in contrast to Colcombe et al. (2006). We did not observe any localized differences when using TBSS in contrast to Tseng, Gundapuneedi, et al. (2013). We did not observe any significant differences or differences in rate of change in volume or cortical thickness ROIs.

Our standard deviations on the MRI data show wide variations in the sample as previously seen in Raz et al. (2010, 2013). Our study accounted for many factors by using groups that were statistically similar in age, sex, education, IQ, depression levels, adherence to Mediterranean diet, and potential for fitness, so the source of these individual differences must be in other unexplored factors that remain to be established.

Our selection of controls may have also played a big role in the lack of substantial effects observed in our study. Whereas previous studies explicitly used sedentary controls (Tseng, Gundapuneedi, et al., 2013; Tseng, Uh, et al., 2013; Winker et al., 2010), we used non-sedentary controls.

Our sample also had high mean IQs and high mean education; these two factors may be protective factors, giving more cognitive reserve in both groups (Alexander et al., 1997; Stern, Alexander, Prohovnik, Mayew, & Stern, 1992; Tucker & Stern, 2011), which may have contributed to why we did not see much change in both groups over the 1-year period.

While the numbers in our groups are not large, they are necessarily so since recruitment of our specific population of supervets limited the number of people that qualified for our study; our numbers compared favorably with the group sizes of Tseng, Gundapuneedi, et al. (2013) and Tseng, Uh, et al. (2013) who reported results from 10 athletes and 12 controls and 12 athletes and 12 controls respectively.

D.5 Conclusion

We comprehensively evaluated the relationship of long-term high-effort endurance exercise on brain structure, but in contrast to previous studies and interventions found very little differences comparing to a non-sedentary control group. This overall lack of substantial differences between supervets and controls provides further evidence that with an already healthy lifestyle, elevating exercise to extreme levels does not provide much brain structural benefit.

The difference we did observe though may manifest a cognitive benefit over the longer term; it is possible a differential between groups may manifest over the 7th and 8th decades, but it is not observable in the 12-month period tested here.

Overall, those participating in long-term high-effort endurance exercise, when compared to moderately active volunteers similar in age, sex, IQ, education, depression levels, adherence to Mediterranean diet, social network, and frequency of cognitive activities, acquired very little substantive improvements in brain structure from their more extreme exercise regime.

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E. General Discussion

Exercise in older adults in the short to medium-term time scale has been previously shown to confer cognitive (Angevaren et al., 2008; Barnes et al., 2003; Chang et al., 2012; Colcombe & Kramer, 2003; Etnier et al., 1997; Hindin & Zelinski, 2012; Middleton et al., 2008; P. J. Smith et al., 2010; Weuve et al., 2004) and brain structural (Bherer et al., 2013; Colcombe et al., 2003; Colcombe, Erickson, Scalf, Kim, Prakash, Mcauley, et al., 2006; Erickson et al., 2009, 2011; Hayes et al., 2013; Ruscheweyh et al., 2011) advantages. Whether these advantages continue or are increased in long-term exercise in older adults has not been thoroughly explored yet; to our knowledge cross-sectional studies have only compared long-term older endurance athletes with sedentary participants whilst not taking into account other lifestyle factors that may have an effect on cognition and brain structure themselves (Tseng, Gundapuneedi, et al., 2013; Tseng, Uh, et al., 2013; Winker et al., 2010). There have not been studies comparing older long-term endurance athletes with non-sedentary participants.

My study aimed to fill these gaps by comparing older adults participating in long-term high-effort endurance exercise, supervets, to non-sedentary older adults that were similar in other lifestyle factors. There were three main aims of the study. The first was to investigate whether long-term high-effort endurance exercise conferred benefits in memory performance, particularly prospective memory performance. This was addressed by including prospective memory measures as well as episodic, incidental, and working memory measures along with a subjective questionnaire on prospective and retrospective memory performance in the behavioral testing session. The second was to explore whether long-term high-effort endurance exercise conferred benefits in performance on attentional measures. This was addressed by including visual search, sustained attention, and attentional control measures in the behavioral battery. The third aim of this research was to investigate whether long-term high-effort endurance exercise conferred benefits in brain structure and whether these

benefits, if any, correlated with enhanced cognitive performance. This was addressed by acquiring volumetric and DTI scans on a subset of our participants.

In terms of similarities of our groups, both were statistically similar on measures of age, sex, education, IQ, depression levels, and potential for fitness. In terms of lifestyle factors both groups were also statistically similar in frequency of cognitive activities, social network, and adherence to Mediterranean diet.

Physiologically, our supervet group was indeed more physically fit than our control group, as demonstrated by less percentage body fat in all supervets and stronger hand-grip in supervet males.

Cognitively, there were surprisingly few differences between our two groups of supervets and non-sedentary controls. There were no differences between supervets and controls in simple reaction time (SRT), speed of processing (Trails A and Symbol Copy), and executive function (Trails B, DSST, and COWAT).

E.1 Supervets vs. Controls in Memory Tasks

Exploring memory tasks specifically in Article I (Paper B), I observed that supervets did not differ from controls in episodic memory, working memory, or incidental memory.

For prospective memory, in a non-focal PM task, supervets showed greater cost of carrying a PM intention than controls at the initial time point, but this difference was not sustained at follow-up. Also in a focal PM condition of another PM task, supervets displayed an abnormal cost of carrying the focal PM intention, while controls did not. At follow-up supervets displayed a larger cost than controls for the focal PM condition.

E.2 Supervets vs. Controls in Attention Tasks

Exploring attention tasks specifically in Article II (Paper C), I observed that supervets did not differ from controls in visual search. On more complex attention tasks, differences emerged.

In the Stroop-switch task, at the initial time point, supervets did not show a normal reaction time cost when switching from color-naming trials to word naming trials, while controls did. However at follow-up, supervets displayed more errors in color-naming trials relative to word-naming trials.

In the RVIP task, there may also have been a differential strategy between the groups. Supervets started the task with quicker and more accurate responses but then adjusted to a slower pace that they could maintain. Controls on the other hand seemed to pace themselves from the beginning, having a shallower fatigue curve, while maintaining similar levels of accuracy until a significant drop off at the end.

E.3 Supervets vs. Controls in Brain Structure

Exploring brain structure using anatomical and DTI scans in Article III (Paper D), I observed very little difference between supervets and controls. There was one difference and only at the follow-up time point: supervets had higher overall white matter axial diffusivity than controls. This can be interpreted as supervets having greater neural integrity and showing less age-related decline. However this difference was not localized and so is still hard to interpret. Also this difference was not correlated with any differences in cognitive performance, and so at the latest time point this difference does not seem to be conferring any cognitive advantages.

E.4 Reserve

Cognitive reserve is the idea that individuals are able to still function well cognitively, in spite of aging or pathology (Stern et al., 1992). Along with cognitive reserve comes the notion of brain reserve, where quantitatively-measured brain indices, such as neuronal count, are

related to preserved brain function allowing for greater age- and disease-related pathology before reaching a threshold where cognitive deficits are apparent.

A number of factors have been linked to cognitive reserve, but in research it is usually operationalized as years of education, IQ, and performance on a vocabulary test (Tucker & Stern, 2011). There must be factors other than these responsible for cognitive reserve and that may build cognitive reserve. These factors could be purpose in life (Boyle et al., 2012; Boyle, Buchman, Barnes, & Bennett, 2010) or the lifestyle factors previously mentioned, frequency of cognitive activities (Christensen & Mackinnon, 1993; Hultsch et al., 1993, 1999; Lachman et al., 2010; Marquine et al., 2012; Schinka et al., 2010; Wilson et al., 1999, 2003, 2005), social network (Bickart et al., 2011; Crooks et al., 2008; Kanai et al., 2012; Seeman et al., 2001), adherence to a Mediterranean diet (Panagiotakos et al., 2007; Tangney et al., 2011), or the focus of my study, exercise.

E.5 Mechanisms

Davenport, Hogan, Eskes, Longman, & Poulin (2012) try to link physical fitness from exercise with increased cognitive function via vascular mechanisms: increased CBF and increased ability for cerebral blood vessels to respond to increased metabolic demand from chemical, mechanical, or neural stimuli. With these vascular mechanisms in addition to increased oxygen saturation and angiogenesis (Lojovich, 2010), I expected my supervets to have better cognition, especially for attentional control tasks since increased CBF has been related to better performance in a Stroop task (Lucas et al., 2012). This may have been the case at the initial time point for the Stroop-switch task, but this was not sustained at follow-up. Other explanations for this result are discussed later.

Brain plasticity from exercise, increasing neurotransmitter concentrations as well as up-regulating receptors and BDNF (Lojovich, 2010; Molteni et al., 2002), should have given supervets more cognitive plasticity and therefore greater processing speed. However, this

greater processing speed was not apparent in our speed of processing tasks. The previously observed benefit of increased BDNF levels being related to better recall (Komulainen et al., 2008) was also not apparent in our memory tasks.

As for the indirect mechanism that exercise may reduce decline, both groups did not have or develop cardiovascular disease, cerebrovascular disease, or diabetes which are all associated with poorer cognitive function (Anstey & Christensen, 2000). Both groups were also similar in depression levels, of which higher levels have been previously observed to relate to greater cognitive impairment (Ganguli et al., 2006), poorer performance on cognitive tasks (McDermott & Ebmeier, 2009), as well as less hippocampal volume (Videbech & Ravnkilde, 2004). Therefore these indirect mechanisms were non-factors in any cognitive differences I could have observed.

E.6 Non-biological factors that modulate performance

The idea behind brain training and cognitive interventions is to give strategies to participants (Verhaeghen et al., 1992) or have participants through practice acquire task-relevant knowledge including discovering the most efficient strategies for a task (Lövdén et al., 2010). Some strategies then are less efficient than others. When approaching a task then, what factors affect the strategy one chooses?

In PM tasks, importance of the task modulates how much one engages the strategy of monitoring, expending attentional resources , regardless of whether or not the task requires it (Gilles O. Einstein & McDaniel, 2005; Kliegel et al., 2004; McDaniel & Einstein, 2000). Performance in identifying the PM cue increases if it is necessary but does not improve if it is not required.

In attention tasks, both intrinsic motivation and interest seem to positively modulate how much effort, again in the form of attentional resources, one is willing to expend in performing

a task (Bunce & Sisa, 2002; Deaton & Parasuraman, 1993; Tomporowski & Tinsley, 1996; Yeh & Wickens, 1988).

All these aforementioned factors all modulate the strategy of how much attentional resources one puts into performing a task. They are all very much related to each other. If one has more intrinsic motivation and interest in a task, he or she will see it as more important and therefore put in more effort.

In addition, the ability to gauge one's own effort and modulate it is interesting. From the sports literature, elite marathon runners' ability to engage this cognitive strategy for stable running performance (Morgan & Pollock, 1977), if crossed over to cognitive task performance would mean greater metacognitive awareness and attentional control.

E.7 Executive summary

Supervets and controls were similar in all lifestyle factors measured and so may have built the similar cognitive reserve from these factors. These lifestyle factors were not explicitly accounted for in other studies so this may be the reason why in my study in contrast to others, I found very little differences in my comprehensive cognitive and brain structural comparisons.

On the PM tasks, the results indicated that supervets may have been self-imposing a higher importance on their performance and therefore engaged monitoring more than controls, expending more attentional resources, regardless of task-necessity. However this monitoring did not result in improvement on task performance.

The results from the Stroop-switch task reveal that supervets may have been using an anticipation strategy in an attempt to be faster. At the initial time point this strategy resulted in better performance, but at follow-up it instead resulted in more errors.

The results from the RVIP, reveal that it may actually be the case that supervets have better metacognitive awareness and are able to gauge their effort and have the attentional control to modulate their effort for sustained performance, while controls cannot and do not do this.

Supervets did seem to place more importance on their performance. They were definitely more motivated to come to our cognitive and MRI sessions and were keen on returning for additional sessions. They truly believed that their long-term high-effort endurance exercise conferred benefits to their cognition among other things.

Structurally, the higher white matter axial diffusivity in supervets indicating greater neural integrity may be a type of brain reserve capacity that is not beneficial cognitively in the short-term but may be beneficial in the longer-term as more age-related decline in cognition occurs.

E.8 Implications

Some may believe that exercise is the panacea to good cognitive health. In fact in terms of overall health benefit, physical activity recommendations from the World Health Organization (WHO), Canada, and the US call for at least 150 minutes per week of moderate-to-vigorous exercise in at least 10 minute bouts, which only 15.4% of Canadians and only 13.1% of Canadians over 60 meet (Colley et al., 2011). Deriving from leisure activity measures from the PASE (Washburn et al., 1993) our controls may have also failed to meet these recommendations at the initial time point.

At least for cognitive health then, a more holistic view must be taken including all lifestyle factors that affect cognitive function. The key to good cognitive health in older aging would then be to participate more frequently in cognitive activities, have larger social networks, adhere more to Mediterranean-style diets, and to exercise a bit but not necessarily to the level of an athlete.

It terms of physical activity, looking at leisure activities derived from the PASE again, our supervets and controls spent a similar amount of time on average walking, doing light sport, and moderate sport. Cognitively, this amount of physical activity along with physical activity from household chores and work may be enough to see a benefit. The threshold of how much and what type of physical activity is enough remains to be seen, as it is beyond the scope of this study.

E.9 Limitations

Our sample had high mean IQs and high mean education; these two factors may be protective factors, giving more cognitive reserve in both groups (Alexander et al., 1997; Stern et al., 1992; Tucker & Stern, 2011), which may have contributed to why I did not see much change in both groups over the one-year period.

While the numbers in our groups are not large, they are necessarily so since recruitment of our specific population of supervets limited the number of people that qualified for our study; our numbers compared favorably with the group sizes of Tseng, Gundapuneedi, et al. (2013) and Tseng, Uh, et al. (2013) who reported results from 10 athletes and 12 controls and 12 athletes and 12 controls respectively.

The fact that there are so few older adult long-term athletes made it very difficult to recruit for our study; only by recruiting larger groups can we confirm the results we have obtained. To mitigate concerns, it should be pointed out that the use of a longitudinal design improved the power of our results and the consistency of these null results across the two time points lends further credence to our assertion that these populations don't differ at this point in time.

We acknowledge that a longer time interval may have exposed differences in cognitive performance and brain structure, but due to our limited time frame for the study, this was not possible.

The multi-dimensional nature of our measures all converge on the same outcome, i.e. no differences, allowing us to have some confidence in our conclusions. Finally, it should be recognized that we used the best statistical techniques to optimize our confidence in the outcomes we recorded including the use of transformations and the robust techniques of bootstrapping and trimmed-means mixed ANOVAs when necessary.

I measured physical activity using a questionnaire, but it has become more common for recent interventions and epidemiological studies to use accelerometers for a more objective assessment (Guiraud et al., 2012; Motl, McAuley, & Dlugonski, 2012; Opdenacker, Boen, Coorevits, & Delecluse, 2008). The accelerometers allow one to monitor the number of steps one takes as well as the intensity level of their activity. The only drawback to accelerometers is that they cost money.

My measures of physical fitness, percentage body fat and hand-grip strength, did not include a measure of aerobic physical fitness. Others have used VO_2 max, or maximal oxygen uptake capacity, as well as other physiological measures (Young, Angevaren, Rusted, & Tabet, n.d.) to directly measure aerobic physical fitness.

E.10 Future Directions

In the future it would be logical to follow-up the current cohort again. In this manner I might be able to observe whether any cognitive benefits of supervets' higher white matter axial diffusivity become apparent.

Another useful future direction would be to recruit from another region of the UK or another country where the mean IQ and education levels are lower and therefore participants would have less cognitive reserve. In this manner I may be able to observe any differential effect from the additional brain reserve in supervets sooner or be able to observe if the contribution of

exercise to cognitive reserve would be more substantial when there is less contribution from education and IQ.

Alternatively we could also recruit from a wider area in our region, perhaps with a mobile setup for the cognitive battery so that participants wouldn't need to come to our laboratory. This would boost our numbers addressing our limitation of sample size and perhaps give us more variation in education and IQ as well.

If the funding allowed in the future, it would be good to then use accelerometers to measure physical activity instead of our current subjective questionnaire-based measure. This would allow for more precision and objectivity in this measure.

In line with this, I would also use VO_2 max as a measure of aerobic physical fitness or a measure that predicted VO_2 max, like the step test (Petrella, Koval, Cunningham, & Paterson, 2001). This would give me another objective measure of physical fitness to compare our groups on and further show that their fitness levels are indeed different.

Future studies could also measure CBF using Doppler. The measurement of CBF would allow me to observe if there is a difference between groups in this measure that supposedly modulates cognitive differences (Lucas et al., 2012). If there is a difference in CBF and still no substantial cognitive differences, then this could be seen as cerebrovascular reserve that will have a benefit later on. If there is no difference in CBF between my groups, then perhaps the amount of exercise my controls take part in is enough to increase CBF and the additional exercise my supervets take part in does not give additional benefit here either.

In the future, the independent effect of effort must be accounted for in task performance. To do this, I could manipulate effort via instruction or have an explicit index of effort in performing a cognitive task. This would ensure that I could derive a measure of the effect of

long-term high-effort endurance exercise on cognitive performance uncontaminated by motivational differences.

E.11 Conclusion

In conclusion, and in contrast to earlier studies looking at short-term exercise interventions and at habitual moderate exercise, our comprehensive evaluation of long-term high-effort endurance exercise suggests a different relationship with cognition and brain structure. Logically, this makes sense because the beneficial effects of exercise cannot be continuous; there must be a ceiling beyond which additional exercise does not confer additional cognitive benefit. In fact the results of this study show clearly that supervets participating in long-term high-effort endurance exercise display only small cognitive and brain structural differences relative to non-sedentary volunteers with a similar age, social, cognitive and neuropsychiatric profile. Cognitively, these small differences manifest in tasks requiring application of effort and strategy, and potentially index differences in effort, metacognitive awareness, or motivation in the supervets. In terms of brain structure, the difference we observed may manifest a cognitive benefit over the longer term; it is possible a difference between groups may manifest over the 7th and 8th decades, but it has not been visible in the 12-month period tested here.

Overall, the current work suggests that the benefits of long-term high-effort endurance exercise in the over 60s may be limited to increased physical fitness and strength, and may not extend to substantive improvements in cognitive performance or brain structure, when comparison is relative to moderately active volunteers of similar age, sex, IQ, education, diet, social, cognitive, and neuropsychiatric profile.

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Appendices

1. Ethical approval

1.1 C-REC

University of Sussex

Life Sciences & Psychology Cluster based Research Ethics Committee CERTIFICATE OF APPROVAL	
Reference Number:	JRJY0111
Title of Project:	Cognitive changes due to long term exercise
Principal Investigator:	J. Rusted
Student:	Jeremy Young
Collaborators:	Naji Tabet
Duration of Approval (not greater than 4 years)	12 months
Expected Start Date:*	February 2011
This project has been given ethical approval by the Life Sciences and Psychology Cluster based Research Ethics Committee (C-REC).	

*NB. If the actual project start date is delayed beyond 12 months of the expected start date, this Certificate of Approval will lapse and the project will need to be reviewed again to take account of changed circumstances such as legislation, sponsor requirements and University procedures.

Please note and follow the requirements for approved submissions:

Amendments to protocol.

- Any changes or amendments to approved protocols must be submitted to the C-REC for authorisation prior to implementation.

Feedback regarding the status and conduct of approved projects

- Any incidents with ethical implications that occur during the implementation of the project must be reported immediately to the Chair of the C-REC.

The principal investigator is required to provide a brief annual written statement to the committee, indicating the status and conduct of the approved project. These reports will be reviewed at the annual meeting of the committee. A statement by the Principal Investigator to the C-REC indicating the status and conduct of the approved project will be required on the following date(s):

December 2011,
2012…….

Authorised Signature	Paul Gard (deputy chair)
Name of Authorised Signatory (C-REC Chair or nominated deputy)	**Paul Gard**
Date	11.02.2011

1.2 C-REC Extension

From: Richard de Visser
Sent: 07 September 2012 12:13
To: Jennifer Rusted
Cc: c-recpsysci
Subject: RE: extension to JRJY09: Cognitive change due to long term exercise

Dear Jenny

I am happy to grant an extension of ethical approval for the project with Reference Number JRJY09 until 31 August 2013.

Pennie, can you please update our records

Best wishes
R de V

Dr Richard de Visser
School of Psychology
University of Sussex
Falmer BN1 9QH
United Kingdom

Term-time drop-in office hours Monday 10-11 and Wednesday 10-11

Psychology for Medicine: <http://www.uk.sagepub.com/books/Book231817>
European Men's Health:
<http://ec.europa.eu/health/population_groups/docs/men_health_report_en.pdf>

1.3 BSMS

Brighton and Sussex Medical School
28/05/2012

Medical Teaching Building
University of Sussex
Falmer
Brighton
BN1 9PX

Professor Jennifer Rusted
School of Psychology
University of Sussex
Falmer
Brighton
BN1 9RH

Dear Professor Jennifer Rusted

Full Study Title: Structural and Cognitive Changes due to Long Term Exercise

R&D Ref No. : 12/080/RUS

I am writing to inform you that the Brighton and Sussex Medical School Research Governance and Ethics Committee (RGEC) which met on **Monday 14th May 2012** has now assessed your application and granted **Research Governance Approval** to proceed with the above named project.

This letter acknowledges that you have all the necessary internal and external regulatory approvals. The sites covered by this approval include:

- University of Sussex (CISC)

Conditions of Approval

The approval covers the period stated in the Research Governance & Ethics Committee (RGEC) application and will be extended in line with any amendments agreed by the RGEC. Research must commence within 12 months of the issue date of this letter. Any delay beyond this may require a new review of the project resources.

Amendments

Project amendment details dated after the issue of this approval letter should be emailed to RGEC for formal approval.

ICH-GCP Monitoring

The Medical School has a duty to ensure that all research is conducted in accordance with the Research Governance Framework and to ICH-GCP standards. The R&D Department will take responsibility for the ongoing monitoring of the study and reporting of any adverse events. In order to ensure compliance the department undertakes random audits. If your project is selected you will be given 4 weeks notice to prepare all documentation for inspection.

I wish you luck with your project and would grateful if you could inform me when the project is completed.

Yours sincerely

Professor Kevin Davies Chair of the BSMS Research Governance and Ethics Committee

2. Consent forms

2.1 Behavioral

University of Sussex

School of Psychology

The long-term cognitive and physiological effects of exercise on healthy older adults.

Dear Participant

You are invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
We are investigating cognitive and physiological differences due to a long-term history of exercise. We are interested because exercise has been implicated in slowing down or preventing cognitive decline associated with ageing. In this study, we want to quantify differences in those with a long term history of exercise and those without in a detailed manner using cognitive and biometric measures.

Why have I been chosen?
You have been chosen because when we sent out a flyer, you expressed an interest in helping out with our research project.

Do I have to take part?
No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

Screening:
You will be asked, first of all, to complete a general medical health screen (that will include calculation of your weight, a measure of blood pressure). Second, you will be asked to provide a buccal swab from the inside cheek of your mouth, which will then be further analysed elsewhere to determine your APOE gene type.

Due to rigorous anonymisation procedures, please note that no person involved with the experiment will ever be in a position to know or reveal your gene type either to you or to another body.

You will be invited to the School of Psychology, University of Sussex to complete a set of standard cognitive tests. The whole session will last about 3 hours.

What do I have to do if I want to take part?
Please note that you must fulfil following criteria if you want to take part in the study: You must be…
60 to 85 years old
English as mother-tongue
Non-smoker (never smoked or not smoked for 5 years)
Weight within the normal range (Body Mass Index between 18 and 30)

You cannot participate in the study if you:
Have high blood pressure/hypertension (systolic > 140 or diastolic > 90)
Have a history of high blood pressure or heart problems
Have a history of psychiatric problems
Are a regular user of cannabis
Are currently being treated for any psychological or physical condition (including use of inhalers)

The tasks you will perform during your visits are routine tasks that we use frequently that measure mental agility, subjective experience, and physiological changes (e.g. blood pressure) over the session.

It is important that for any testing session, you do NOT
drink any beverage containing caffeine for two hours before the session starts
have any alcohol or any other psychoactive substance on the day of the session before you come to be tested.

Please note that we are NOT looking at your individual performance but at the performance of the entire group of volunteers. All data will be anonymised at collection.

What are the other possible disadvantages and risks of taking part?
None.

What are the possible benefits of taking part?
There are no immediate benefits to you in taking part; however you will be helping research involved in understanding how exercise affects mental agility and physiological measures. The results will be used to develop models that can benefit clinical projects as well as develop theoretical models of change over the lifespan.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer should be addressed to the researchers in the first instance (contact details in section 11). You will be advised on how you can take your complaint further, should you so wish.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be anonymised and kept confidential. We maintain strict levels of confidentiality with all collected data. In addition, in this study procedures are designed to ensure that both the researchers and the volunteers remain blind to the genetic makeup of the individuals taking part. Therefore your saliva sample will be assigned a random code identifier, and no person involved with the experiment will be in a position to know or reveal your genotype either to you or to another body.

Contact Details for members of the research team:
Jeremy Young, School of Psychology, University of Sussex, Brighton, BN1 9QH (j.young@sussex.ac.uk), Tel: 01273 872776
Prof Jennifer Rusted, School of Psychology, University of Sussex, Brighton, BN1 9QG, (j.rusted@sussex.ac.uk), Tel: 01273 678325
Dr Naji Tabet, Brighton and Sussex Medical School, University of Brighton, Brighton, BN1 9PX, (n.tabet@bsms.ac.uk), Tel: 01273 644503
Nicolas Farina, School of Psychology, University of Sussex, Brighton, BN1 9QH (n.farina@sussex.ac.uk), Tel: 01273 872776

University of Sussex

School of Psychology

CONSENT FORM

Title of Project: The long-term cognitive and physiological effects of exercise on healthy older adults.

Research team:

Jeremy Young, School of Psychology, University of Sussex, Brighton, BN1 9QH (j.young@sussex.ac.uk), Tel: 01273 872776

Prof Jennifer Rusted, School of Psychology, University of Sussex, Brighton, BN1 9QG, (j.rusted@sussex.ac.uk), Tel: 01273 678325

Dr Naji Tabet, Brighton and Sussex Medical School, University of Brighton, Brighton, BN1 9PX, (n.tabet@bsms.ac.uk), Tel: 01273 644503

Nicolas Farina, School of Psychology, University of Sussex, Brighton, BN1 9QH (n.farina@sussex.ac.uk), Tel: 01273 872776

	Please initial box
I confirm that I have read and understand the information sheet dated December 2010 (Version 1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason. I understand that if I withdraw for the study, all of my data will be removed from the study.	☐
I understand that data collected during the study will be anonymised at collection, but that the anonymised data may be looked at by all members of the research team now and in the future.	☐
I agree to take part in the above study.	☐

_____	_____	_____
Name of volunteer	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_ _____
Researcher	Date	Signature

When completed, 1 for volunteer; 1 for researcher site file

brighton and sussex medical school

2.1 MRI

Structural and Cognitive Changes due to Long Term Exercise

Dear Participant

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1

What is the purpose of the study?

We are investigating cognitive abilities (that is, the ability to perform tasks requiring mental agility) and structural differences in the brain associated with long-term exercise. We are interested because exercise has been implicated in slowing down or preventing decline in mental agility associated with normal ageing. In this study, we want to quantify differences in those with a history of long term exercise and those without in a detailed manner using brain imaging and cognitive measures. We also want to see if there are differences in the changes in the brain between these two groups in the course of a year.

Why have I been chosen?

You have been chosen because you came along to complete a series of cognitive tasks earlier in the year.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

You will be invited to the Clinical Imaging Science Centre (CISC) for an MRI scan that will take approximately 30 minutes. You should allow an additional 30 minutes for preparation – so the total session will be one hour.

What do I have to do?

You will not need to do anything besides come to the MRI scan session. You will also have an opportunity to come back 1 year later for the same scan to see if there are any changes.

It is important that for any scan session, you do NOT

- drink any beverage containing caffeine for two hours before the session starts
- have any alcohol or any other psychoactive substance on the day of the session before you come to be tested.

What are the side effects of any treatment received when taking part?

If we find anything unexpected or potentially abnormal on the imaging scan, we will inform you and suggest you see your GP for further advice.

What are the other possible disadvantages and risks of taking part?

There are no known disadvantages or risks of taking part. The images that will be acquired are not for diagnostic purposes and the examination should not be considered an alternative to a proper medical consultation. However, very rarely something may be found in the images and an expert opinion sought. If there are any unexpected findings that need further analysis, your GP will be contacted in the first instance. The GP will then contact you if further tests are required. If you have any concerns about this, please contact a member of staff.

What are the possible benefits of taking part?

There are no immediate benefits to you in taking part; however you will be helping research involved in understanding how exercise affects brain structure and how it is related to mental agility. The results will be used to develop models that can benefit clinical projects as well as develop theoretical models of change over the lifespan.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

11. Contact Details:

Jeremy Young, School of Psychology, University of Sussex, Brighton, BN1 9QH (j.young@sussex.ac.uk), Tel: 01273 872776
Prof Jennifer Rusted, School of Psychology, University of Sussex, Brighton, BN1 9QG, (j.rusted@sussex.ac.uk), Tel: 01273 678325
Dr Naji Tabet, Brighton and Sussex Medical School, University of Brighton, Brighton, BN1 9PX, (n.tabet@bsms.ac.uk), Tel: 01273 644503

Part 2

What if there is a problem?

It is not anticipated that any problems will occur. However, if you do have any concerns, they should be addressed to the researchers in the first instance (contact details in Part 1 section 11). You will be advised on how you can take your complaint further, should you so wish.

Complaints

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions, 01273 872776.

Harm

The University of Sussex has insurance in place to cover its legal liability should any harm arise from this study.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential.

What will happen to the results of the research study?

The results of the research study will be used in group analyses and written up for publication in a scientific journal.

Who is organising and funding the research?

The research is funded jointly by the Ageing Research Centre, Sussex, University of Sussex and the Sussex NHS Partnership Trust

Thank you for taking the time to read this information sheet.

brighton and sussex medical school

CONSENT FORM

Title of Project: Structural and Cognitive Changes due to Long Term Exercise

Names of Researchers:

Jeremy Young, School of Psychology, University of Sussex, Brighton, BN1 9QH (j.young@sussex.ac.uk), Tel: 01273 872776
Prof Jennifer Rusted, School of Psychology, University of Sussex, Brighton, BN1 9QG, (j.rusted@sussex.ac.uk), Tel: 01273 678325
Dr Naji Tabet, Brighton and Sussex Medical School, University of Brighton, Brighton, BN1 9PX, (n.tabet@bsms.ac.uk), Tel: 01273 644503

I confirm that I have read and understand the information sheet dated **April 2012 (Version 1)** for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
I understand that if there are unexpected findings that need further investigation you will, with my consent, inform my GP who will notify me if further tests are needed.	☐
I agree to take part in the above study.	☐

_____	_____	_____
Name of Volunteer	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_ _____
Researcher	Date	Signature

When completed, 1 for participant; 1 for researcher site file.